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Studies on the Biology and Taxonomy of Soil Algae.

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INTRODUCTION

By soil algae I mean the algae growing on and in the earth. In the present work I have not dealt with the algal flora of rocks, stones, moss tufts, and the like. In nature it may be difficult to delimit sharply the algal community of the soil. As an attempt at a somewhat more precise definition of the term I give below a tentative division of the algae according to their habitats. Of course many species occur in various habitats and can, accordingly, be referred to several of the groups mentioned.

A. Aërial algae, or such as grow out of water.

1. Eu-aërial algae occurring on prominent objects such as palings, large stones, dry rocks, tree-trunks, walls, and drawing moisture exclusively from the atmosphere in the form of rain or dew; they are, therefore, very often exposed to prolonged and intense droughts. In our climate the chief representatives of this group are the pulverulent green algae *Desmococcus*, *Apatococcus*, and *Pleurococcus*, as well as the filamentous form of *Prasiola crispa*, and *Stichococcus bacillaris*, *Chlorella ellipsoidea*, *Trebouxia arboricola*, *Trentepohlia* etc.
2. Terrestrial algae comprising the species growing on soil of all kinds. These species must be capable of surviving in some way or other a more or less intense desiccation, though it will never be so intensive as that undergone by the aërial algae, for the soil will be much better off as regards the formation of dew, in part internal, and in part external, than the prominent objects. The store of moisture always present in the deeper layers of the soil will thus be able to

benefit even the algae growing at the surface (see below, p. 103). FISCHER (1926) has alleged that the terrestrial microflora is recruited from two sources, partly from the aërophiles and partly from the hydrophiles, and accordingly he rejects the possibility of a separate terrestrial biocoenosis. True, it must be conceded that a great number of the terrestrial algae may also occur in aërial or hydrophytic habitats, but how can it be known which was the original habitat of the species, and to which it has spread later on?

Since, however, there are numerous transitional cases between the terrestrial algae and the aërial as well as the hydrophytic algae, I will divide the terrestrial algae into three subordinate groups.

a. Aëro-terrestrial I will term such algae as often occur in aërial localities, but are found growing on the ground as well. Among these are e. g. *Prasiola crispa*, which in the Hormidium form frequently occurs in purely aërial situations on tree trunks, but will also spread over the ground at the foot of the tree.

b. Eu-terrestrial species are such as normally grow in or on soil only moistened by atmospheric water. Among the eu-terrestrial species I also include the bryophilous Diatoms of the xerotic type (BEGER 1927). These are in reality found on the surface of the soil and only exceptionally on top of the mosses themselves. The eu-terrestrial algae may tentatively be divided into two groups:

  x. Epiterranean species met with on the surface of the soil. Some of them are only known from this situation, as *Vaucheria*, *Prasiola*, *Botrydium*, while others may also be found at greater or lesser depths below the surface.

  xx. Subterranean species occurring below the surface of the soil and hence developing in cultures of soil samples from the deeper layers. All subterranean algae known so far are able to grow on the surface of the soil, in the light, and only attain a vigorous development there. They must therefore be regarded as facultatively subterranean. The existence of obligately subterranean algae must be questioned; at any rate, up to the present none are known.

c. Hydroterrestrial algae grow on soil which is always damp to wet, because the level of the ground water is always near the surface. The species belonging to this group may also be met with now and then in the company of the euterrestrial species. Among these must be included a large number of Diatoms. Further, localities with hydroterrestrial algae fall into two sub-sections according as the water is salt or fresh. The salt soil community is especially met with in littoral meadows where *Rhizoclonium*, *Vaucheria* and *Lyngbya* species frequently form extensive growths.

The freshwater community occurs in bogs, on low-lying heaths and in meadows and is represented by *Coccomyxa*, *Mesotaenium*, *Desmidiaceae*, *Cyanophyceae*, and *Diatomeae*. *Zygogonium ericetorum*, too, should probably be included among these.

3. Pseudo-aërial algae grow on rocks and in other localities receiving a special supply of water, such as spray from waterfalls, springs, and the like. Among these I include the algae in the "black stripes" on rocks, and the algae of slopes and cliffs watersoaked from springs. The environment of these algae, provided the water supply is fairly constant and plentiful all the year round, very closely resembles that of the hydrophytes; at its other extreme the environment of this community may, however, also come very near to that of the eu-aërial community, viz. when the amount of water is very restricted and entirely disappears in the summer. Bryophilous Diatomeae of the mesophilous type must probably also for the most part be classed among these algae (BEGER 1927).

B. Hydrophytic algae, or such as grow submersed in water, in lakes, streams, and the sea.

When the term soil algae is used in the sequel, it refers principally to Eu-terrestrial algae.

I. The Development of our Knowledge of the Soil Algae.

A. Before the Era of Ecology.

Soil algae have been known from very old times, for many *Chlorophyceae* and *Cyanophyceae* form rather conspicuous growths on the surface of the ground and are thus bound to attract attention. As an example I may mention that according to LYNGBYE (1819) the name of *Nostoc* is found in PARACELSUS, who probably meant *Nostoc commune*. This species was, indeed, well known in the middle of the 18th century and was figured by DILLENIUS (1741, Pl. X, fig. 14). That it was known in very early times may also be gathered from the fact that popular names for it occur both in Latin (coeliflos, coelifolium) and in Danish (Skyefald, Troldsmør) (LYNGBYE l. c.). *Prasiola crispa* = *Ulva terrestris* LYNGBYE was already described by KYLLING (1688, p. 105) as "Muscus uvida amans foliis subrotundis expansis —", and is probably the first Danish soil alga mentioned in the literature.

The botanists of the 17th and 18th centuries distinguished few species of soil algae and were unable to separate the algae from the mosses, lichens, and fungi; as a consequence they often had representatives of all these groups within the same genus.

The beginning of the 19th century was marked by a considerable interest in the lower plants. This resulted in the appearance of a comprehensive literature on the algae, too; thus also in Denmark, of which later on. In the course of time not a few species of soil algae were described and figured, and by and by botanists learned to distinguish them from the other groups of cryptogams, and were able to give a natural delimitation to most of the genera. As a result several of the earlier generic names disappeared as names for algae, thus *Tremella* and *Byssus*. I shall here merely mention some of the most important authors: VAUCHER (1803), DILLWYN (1809), AGARDH (1812, 1817), LYNGBYE (1819).

Towards the middle of the 19th century a further impetus was given to algology, and as far as the soil algae were concerned many new species, both of *Chlorophyceae* and *Cyanophyceae*, were described by KÜTZING (1845, 1849, 1845—71), HASSALL (1845), NÄGELI (1849), and RABENHORST (1864—68). KÜTZING especially stated about many species that he had found them on the soil.

Many of the descriptions of these earlier algologists, however, are worded in a way that makes it difficult to understand what was meant by them. It was left to the great monographers from the close of the 19th century to make order out of chaos. They subjected to a critical examination especially the exsiccata preserved in the herbaria, and thus succeeded in attaining clarity as to many of the species. In respect of the *Cyanophyceae* we may here mention the monographs of BORNET et FLAHAULT (1886—1888) and GOMONT (1892). For the *Coccogoneae* we have so far felt the want of a similar monographic elaboration of the material; WILLE began a monograph, but did not manage to finish it (WILLE 1925). GEITLER (1925, 1930—32), however, will in some degree supply the deficiency.

There exists no monograph dealing with the *Chlorophyceae* collectively but various groups have been treated separately, thus the *Desmidiaceae* (W. and G. S. WEST 1904—23), and *Vaucheria* (HEERING 1907, 1921); but notably in the field of the unicellular green algae (*Isokontae* as well as *Heterokontae*) such great advance has been made during the last decades that the pressing need of a general view of the muliplicity of forms is now felt. PRINTZ (1927) is a great help, but cannot of course suffice as regards the species.

EHRENBERG (1843, 1854) was the first to point out the presence of *Diatomeae* on and in the soil. He examined numerous samples of soil from various parts of America, often quite small samples derived from dried plants. In these he found amongst other things a number of algae, and especially the two chief soil Diatomeae, *Hantzschia amphioxys* and *Pinnularia borealis*, described here for the first time. Later on, in his Microgeology (1854) he mentions his examination of cultivated soils from divers parts of the world, and figures (Pl. 34) what he has found in the different samples, amongst other things various Diatomeae. There are figures of *Hantzschia amphioxys* from nearly all localities, and from some of them *Pinnularia borealis*. Thus he has shown that these two species must be assumed to occur nearly everywhere on the face of the earth, and chromatophores being drawn in not a few of the frustules, it must be inferred that the Diatomeae actually lived in the soil. GREGORY (1856) points out the importance of EHRENBERG'S discovery, but otherwise furnishes no fresh contribution to our knowledge of the soil Diatoms. A study of the not inconsiderable Diatom literature from the latter half of the 19th century shows that the systematics of the Diatomeae, the knowledge of the valve structure of the individual species, is the all-engrossing topic, while the interest in their biology is minimal. True, under some few species there are notes stating that they were found "on damp soil", "among mosses" and the like; but such indications are so vague that it is difficult to form any idea from these works, in other respects so accurate and painstaking, as to the distribution of the Diatomeae on the surface of the soil. I may here mention W. SMITH'S Synopsis (1853—56), RABENHORST'S Kryptogamenflora (1864), KÜTZING (1865), VAN HEURCK'S Synopsis (1880—85), Traité (1896). As regards the Naviculeae the whole of the systematic work from this period has been revised and collected in CLEVE'S Monograph (1894—95). For the genus *Nitzschia* there exists a similar revision though not so complete in CLEVE und GRUNOW (1880).

According to KOLBE (1932, p. 258), DEBY (1879) was the first who was aware that there exists a special group of species which live in the main on soil, just as another group of species live among mosses on the ground, on stones, and on tree-trunks. DEBY'S work was recorded by PFITZER in SCHENCK'S Handbuch (1882 II, p. 405); but later on this knowledge was evidently forgotten. In KARSTEN'S elaboration of the Diatomeae in ENGLER und PRANTL, Die natürlichen Pflanzenfamilien (1928) there is, practically, no information about the Diatomeae living in the soil; on p. 111 alone it is stated that they are "über die ganze Erdoberflache verbreitet"; but in the text there is no further reference to the soil Diatomeae.

B. The Era of Ecology.

The close of the 19th century witnessed the rise of ecological points of view and since then the systematic works have as a rule contained fuller information as to the habitats of the species. This information is, however, so scattered throughout the literature that it is difficult to form an idea of the algal flora of the soil as a whole. Since that time more direct work on this flora has also been done along various lines. First, investigations in nature of the algal vegetations visible to the naked eye, or direct microscopical examination of the soil samples collected, have been directed towards establishing the floristic composition of the individual plant communities and the ecological importance of the algae in the communities; secondly, individual species have been subjected to a more detailed investigation in respect of their morphology and biology; thirdly, it has been attempted to advance our knowledge of the soil algae by means of cultures.

1. Floristic-ecological Investigations.

The first attempt to list the species of the soil algae of a plant community as a whole was presumably due to Graebner (1895) who described the heaths of Northern Germany, the development of their vegetation, and the various species of heath vegetation. Here he also includes the algae, to which he ascribes no small importance as the first immigrants on new soil, causing the first formation of humus in the poor soil. Finally, he gives (p. 547) a collective list of the algal species found on the heath. Here he enumerates 31 species of Cyanophyceae, 3 species of Diatomeae, and 18 species of Chlorophyceae, some of which, however, more probably belong to the peat bogs and therefore cannot be regarded as soil algae in the sense in which they are treated in the present work. Graebner also used enrichment cultures to confirm his results in various ways.

Warming (1904) has given ecological descriptions of the natural history of the marshes. Such a community lies on the borderline of our subject, for the soil of these localities is always completely saturated with water. We shall therefore merely make brief mention of Warming's investigations. He has given detailed lists of the Cyanophyceae (determined by Schmidt), and Diatomeae (determined by Østrup) found in this community. Unfortunately for the most part he does not say which of the Diatomeae were living in the localities and which of them occurred as dead frustules only. His lists of species are thus probably far from being a true expression of the Diatom flora living in these situations; many of the species mentioned are no doubt merely valves of marine Diatomeae washed ashore here.

COMÈRE in a series of papers (1906, 1910, 1913) has dealt with the ecology of the freshwater algae. His studies were made in the south of France, in the neighbourhood of Toulouse and the Pyrenees. Special interest attaches to COMÈRE's work owing to what he calls "formations passagères". By this he means ditches, pools, and other small bodies of water, which only contain water for a short time in rainy periods, but dry up entirely in periods of drought. These localities harbour especially a great many Conjugatae, which survive the periods of drought in the form of zygospores; but there also occur a number of algae which must be counted among the soil algae, thus *Hormidium flaccidum* and many of the small *Navicula* and *Nitzschia* species mentioned by him.

In the treatise of 1913 in which he surveys the different formations of freshwater algae and aërial algae, he also has "algues epigées"; but these algae are not discussed in detail.

FRITSCH has treated the algal flora of the tropics, especially of Ceylon, in several papers (1907 I, II, III). He calls attention to the enormous development of the aërial algae in the warm moist climate; still it is not so much the algal vegetation of the soil itself that he deals with, as the vegetation of rocks, tree-trunks, and other prominent objects. Yet according to FRITSCH the algal flora of the soil also would seem to be very rich in the tropics, and it is composed especially of Cyanophyceae, whereas the Chlorophyceae are of small importance. He says nothing about the Diatomeae. In his paper of 1907 II he brings together everything known up to then of the tropical algal flora occurring in freshwaters and on the soil. In 1907 III he discusses the importance of the algae as the first colonists on new soil and their significance for the higher plants which subsequently immigrate there. From various instances known from earlier times (WELWITSCH, WEST) he arrives at the result that a dense covering of algae on the surface of the ground on very dry and sandy soil is able to regulate the moisture of the soil and thus shelter and render possible the growth of phanerogams. FRITSCH also thinks that the algae have an important influence as manure for the soil.

In this connection it would seem appropriate to recall TREUB's (1888, p. 221) well-known observation from Krakatau that the first immigrants to the island after the great eruption in 1883 were blue green algae (*Tolypothrix*, *Anabæna*, *Symploca*, 3 species of *Lyngbya*). They formed a slimy layer on rocks and debris, and must be supposed thereby to prepare the way for the immigration and growth of ferns and higher plants.

FRITSCH and SALISBURY (1915) have given an interesting description of the succession of cryptogams on burnt heath in England. The first immigrants were *Cystococcus humicola* and *Gloeocystis vesiculosa*, *Trochiscia aspera* and *Dactylococcus infusionum*. Various fungi grow in their mucilage envelopes, and by degrees the algae are caught by lichens. Coincidently with the formation of lichen thalli *Mesotaenium violascens* appears in quantity, and in addition *Hormidium flaccidum* and *Zygogonium ericetorum* make their appearance.

The chief biological result emerging from the investigations mentioned above is presumably that the algae develop with especial vigour on "new soil" and prepare the way for other plants. The same observations are to be found somewhat later in BOYE PETERSEN (1915, pp. 283 and 301) and PUYMALY (1924, p. 86). The latter author especially describes how the soil algae, e. g. in a garden walk, are constantly competing with the grass weeds cropping up. Whenever the latter are weeded out, the algae attain a vigorous growth, which is gradually brought to cease by the renewed appearance of the grass.

PIERCY (1917, p. 515) has described a similar competition between *Hormidium flaccidum* and grasses. In very dry periods in the summer the latter die off, whereas *Hormidium* is better able to withstand the drought; in a succeeding damp period the algae will therefore spread extensively. The following spring, however, numerous grass seeds will germinate and form a dense carpet which may choke the algae until after the next summer drought.

In this period the first attempt was also made to erect "life forms" of aërial algae, viz. by FRITSCH (1907 I). Work in that field was carried on later by PUYMALY (1924, p. 111) for the Chlorophyceae.

FRANCÉ's paper "Das Edaphon" (1913) marks an essential contribution to the study of the soil algae. Several new ideas and problems are set forth in this work, even though it cannot be said that they have been solved. By "edaphon" FRANCÉ means all organisms living in the soil; the term is formed after the analogy of "plankton". Among the edaphon are included bacteria, fungi, protozoa, nematodes and other small lower animals. Thus the author has set himself no small task when he proposes to deal *en bloc* with all these groups and their biology. The work is full of good suggestions as to subjects for investigation; but as a rule the investigation itself only amounts to feeble attempts. FRANCÉ has simply taken samples of soil from all kinds of localities and examined them directly under the microscope. Sometimes, however, he has taken counts of the organisms present; no details of the counting method are given. The value of the results of the count is moreover much diminished by the fact that generally he only gives totals per mm³ of soil of all the organisms (minus bacteria); hence information is rarely found as to how many algae there occurred. In a list of "edaphic" organisms 63 algae are enumerated, not a few of which, however, are probably not genuine soil inhabitants. Thus several Diatomeae are undoubtedly included which have only been found as dead valves in the earth, and others which have been found in such moist situations that they cannot be regarded as soil algae in the strict sense. The number of Chlorophyceae is rather low, and among them there are even some Desmidiaceae which are hardly genuine soil inhabitants.

FRANCÉ has also made several investigations on the ecology of the edaphic algae and thereunder touched upon diverse questions which have later been treated more fully or deserve to be so. Thus he has met with Diatomeae as well as Cyanophyceae living below the surface at a depth of 5—15 cm, has tried to clear up the influence of the seasons on edaphon, the effect of the moisture of the soil on the flora and the fauna, the distribution of the edaphon according to the geographical altitude, the influence of the chemical character of the soil, etc. In the sequel I shall repeatedly have occasion to go more fully into this work and its results. The chief interest of FRANCÉ's treatise presumably lies in the fact that an attempt is here for the first time made to give a comprehensive description of the world of soil organisms, and it is pointed out that we have here a separate flora and fauna different from that of the water as well as from that of the aërial localities proper (tree-trunks, palings, rocks etc.).

In my own paper (BOYE PETERSEN 1915) I do not attempt to solve so many problems, but direct most of my efforts towards the correct determination and the morphological examination of the soil organisms. The work falls into two sections, the first dealing with Diatomeae, the second with Chlorophyceae. The Cyanophyceae were disregarded, since SCHMIDT's comparatively new and excellent elaboration (1899) was available for Denmark. I enumerate 36 species of Diatomeae found on the surface of the soil, partly by direct microscopic examination, partly in cultures which will be mentioned later on. Of these 36 species, 5 are described as new, and in addition some new varieties of species already known. On comparing my observations as to the occurrence of these species on soil with ØSTRUP's (1910) observations as to their occurrence in freshwater in Denmark, I can establish the predominant occurrence on soil of some 24 species; 9 species would seem to occur just as often in freshwater as on soil, while 4 must be regarded as freshwater species only occasionally met with on soil. I further point out, first, that virtually all the Diatomeae living on soil are pennate forms having raphes and therefore motile, and, secondly, that they all belong to the small or minute forms. This fact is assumed to be connected with their capacity to withstand drought.

The section on the Chlorophyceae consists in the main of descriptions of the morphological and systematic characteristics of the algae found in nature as well as in cultures. I have also attempted a division of the algae according to their occurrence on soil of different degrees of acidity. Since, however, these determinations have not been made with the exactitude called for nowadays, this part of the work should no doubt be tested afresh.

In continuation of my work comes a paper by PUYMALY (1924) who only mentions the Chlorophyceae. PUYMALY assumes a very critical attitude and treats both the systematics, the morphology, and the ecology of the green algae. I have already mentioned his attempt to erect life-forms for the aërial green algae; he has 5 types in all, 2 of which, "la forme gélatineuse" and "la forme en natte", are regarded by him as characteristic of the algal community of the soil. To the first type belong e. g. *Mesotaenium*, *Cylindrocystis* and *Gloeocystis*, to the second e. g. *Zygogonium ericetorum*, *Tribonema bombycinum*, *Hormidium flaccidum* and the filamentous form of *Prasiola crispa*.

My investigations on Icelandic algae also are worked out (1923 and 1928 I) according to the floristic-ecological method. I here continue my attempt to establish the floristic composition of the algal community of the soil and further (1928 I) I give a tentative ecological division of the Icelandic soils. This division, however, is by no means exhaustive, since the necessary information as to the whole nature of the soil was not available. I think, however, that I am able to show the great dependence of the algal flora on the content of manure in the soil and to establish the markedly nitrophilous nature of a number of species.

2. Investigations on Individual Species.

Some authors, especially English authors, have taken up individual species of terrestrial algae for a more detailed investigation, morphologically as well as biologically. Thus *Zygogonium ericetorum* has been closely studied by WEST and STARKEY (1915) and by FRITSCH (1916). The latter author in collaboration with some of his pupils (FRITSCH 1922 I, FRITSCH and HAINES 1923, FRAYMOUTH 1928) has tried in various ways to throw light on the peculiar physiology of the aërial algae, which is connected with the special environmental conditions under which they live, and notably with the fact that they are always exposed to shorter or longer periods of drought. Thus of the soil algae FRITSCH (1922 I) has studied *Prasiola crispa* (in the *Hormidium* form) and *Zygogonium ericetorum*. FRITSCH and HAINES (1923) have studied these species in more detail and likewise *Hormidium flaccidum* and *Cystococcus humicola*.

FRAYMOUTH (1928) investigated the respiration of *Prasiola crispa*, especially in relation to the conditions of moisture, and compared it with *Trebouxia* sp., *Xanthoria parietina* and *Hypnum* sp. in this respect.

Finally PIERCY (1917) has given an interesting description of the morphology and life conditions of *Hormidium flaccidum*.

We shall return to these works in the sequel.

3. Cultures.

Before the era of ecology attempts had been made to cultivate algae, but these earlier experiments were nearly all of them undertaken almost exclusively for morphological purposes. The essential development of the culture technique, however, only took place in this period, and the purpose of the cultures often became physiological-biological in addition to morphological, the ecological point of view now asserting itself. Hence I mention all investigations based on alga-cultures in this section, no matter whether some of them fall before the era of ecology, and irrespective of the fact that several of them only deal with the morphology and systematics of the algae.

The various culture methods employed and which are each applicable for various purposes are

a. Enrichment cultures, especially suitable for finding out what species are present in the soil and for studies of the morphology and life history of the individual species. By means of the enrichment cultures our knowledge of the algal formations on the surface of the soil has been advanced, just as the existence of the subterranean algal flora has been discovered by these kind of methods. On the other hand, all conclusions of a quantitative kind from enrichment cultures are very uncertain.

b. Dilution cultures, either in liquid or agar dilutions are a considerably better though far from perfect means of finding a numerical expression for the amount of algae in the soil.

c. Unialgal cultures, are cultures in which only one species of alga is present but also other organisms, e.g. bacteria and possibly fungi. They are suitable for systematic-morphological studies of the individual species and their development, as well as for certain physiological studies, especially of the physiology of motility.

d. Finally pure cultures, which are rather difficult to realise, are necessary in all studies on the physiology of nutrition. Numerous are the errors committed by drawing conclusions from algal cultures containing bacteria.

a. Enrichment Cultures.

As already mentioned (p. 7), GRAEBNER (1895) tried to imitate the growth of algae in a natural environment by means of cultures. To flasks containing sterile, washed sand and moistened with sterile water he added soil from heaths, and a copious vegetation of Chlorophyceae and Cyanophyceae then appeared on the sand. In this GRAEBNER saw a reflection of what takes place in nature, though there at a slower rate. JACOBSEN (1910) set up cultures by inoculating liquid nutrient media containing nitrogenous organic substances such as fibrin, albumin, gluten (of wheat-flour) or casein, with soil. A great many decomposition bacteria then developed, but also divers green organisms, especially *Chlorogonium euchlorum*, *Spondylomorum quaternarium*, *Chlamy-*

domonas Ehrenbergii, *C. intermedia*, *C. variabilis*, *Euglena* and the colourless *Polytoma uvella*. These organisms were nearly always present in the soil; but as it was necessary to use a considerable amount of soil in order to be sure of producing them, it must be supposed that they only occurred there in small numbers.

ESMARCH (1911, 1914) adopted quite another method for producing a growth of Cyanophyceae in soil samples. The soil was placed in a layer 1 cm thick in a sterile Petri dish, was covered with a piece of sterile filter paper which was pressed down firmly against the surface of the soil and moistened with sterile water. The dish was then closed and placed in a hothouse. In a great many of the cultures Cyanophyceae grew up after some time. Their filaments pierced the filter paper and on the surface of this formed blue green or brownish green spots. Here it was then possible to determine the species, and in such cultures from various German colonies (1911) he identified 24 species in all, and from Germany (1914) even 45 species of Cyanophyceae. Green algae and Diatomeae also appeared in the cultures; but he has not mentioned what species.

I have not had access to a paper by ROBBINS (1912); but it has often been cited (e. g. by WAKSMAN 1931, pp. 203 and 209). From this it appears that ROBBINS proposed to procure pure cultures of soil algae, but provisionally used an enrichment culture method reminiscent of the one used by GRAEBNER (1895). To sterile flasks containing pure sand moistened with a solution of inorganic salts were added 10 gr of soil. When left to stand in the light a luxuriant vegetation of algae grew up, especially Cyanophyceae, a single Diatom species, and a Chlorophycea. This material he then seems to have used for agar dilutions, and possibly he has produced the aforementioned species in pure culture, but this does not appear clearly from the quotations. ROBBINS only found algae in the cultivated part of the soil in California, but none in uncultivated soil.

For the purpose of elucidating the question whether there were living Diatomeae on and in ordinary soil without any plant covering I collected (BOYE PETERSEN 1915) soil samples in a sterile way in Freudenreich flasks from the surface of the ground in a number of localities in Denmark. The soil was moistened with sterile water and the flasks were placed in the light in a window facing north. In all of them a brownish layer of Diatoms grew up, in some of them also green algae and moss protonema. By this procedure I found several Diatom species which might easily have remained unnoticed by direct examination of the soil samples under the microscope, just as it could likewise be established that these species were living in the soil.

By adding to a sterile mineral salt solution, soil from samples which had been kept for as much as 73 years in closed vessels and placing the cultures in diffuse daylight, BRISTOL (1919) succeeded in showing that living alga germs were present even in the oldest soil samples, and she was able to ascertain the presence of 16 species of algae in these cultures. Since then BRISTOL (ROACH) (1920) has used the same method in investigating the algal flora in English cultivated soils, especially for the purpose of obtaining a flora list.

MOORE and KARRER (1919) used bottles containing sand moistened with a mineral salt solution for similar cultures, to each of which were added 10 gr of soil from various depths, mainly from the Missouri Botanical Garden. In this way they were able to make 17 species of algae develop, and a culture method essentially the same was adopted by MOORE and CARTER (1926) for further studies on the subterranean algal flora in the Missouri Botanical Garden. Altogether 25 species of algae were identified in these cultures; but in addition several species were found which could not at that time be identified.

BOYE PETERSEN (1928 II) used enrichment cultures in investigating the algal flora of soil samples from various plant formations in Iceland, thus in contrast with the English and American investigations for the most part from uncultivated soil. I set up two parallel culture series, each soil sample being divided into two parts, one of which (about 1 c.c. of soil) was added to a sterile liquid mineral medium in Freudenreich flasks, while the rest of the soil samples in the original glass, which was closed with a cotton plug, were moistened with sterile water; all cultures were placed in the light. It then turned out that algae were present in all the surface samples collected. 55 species of Diatoms, 9 species of Cyanophyceae, and 16 species of Chlorophyceae were found. It appeared from the cultures that in these natural localities the moisture of the soil was the chief ecological factor; whereas the snow covering did not seem to have any influence on the number of algal species. All other things being equal, the algal flora was richest in species on the naked ground, whereas it was poorer where the ground was covered with a thick moss carpet or a dense phanerogam vegetation. I made a similar investigation in Denmark (BOYE PETERSEN 1932 I) of soil samples from various more or less natural formations on Hammer-Bakker: heath, beech wood, natural meadow, etc. and for purpose of comparison from a manured pasture in the neighbourhood. Here it turned out that the cultivated (manured) soil contained most alga species, and that the number of species of the algal flora in the natural localities increased with the moisture of the soil. Here again the algal flora proved richest in species on naked soil, poorer on soil covered with withered leaves, moss, or a dense phanerogam vegetation. Quite recently SINGH (1933), too, has worked with enrichment cultures. By means of cultures in the main according to the method of BRISTOL he has obtained an algal flora from Indian soils.

ESMARCH (1914) already attempted by means of his cultures to obtain information as to the frequency of Cyanophyceae in the soils examined. He selected a number of soil samples from the various localities, and tried whether or not they contained Cyanophyceae. From some localities he obtained Cyanophyceae in virtually all the samples, from others not a single one, from others again algae developed from different percentages of the samples. He uses this as a measure for the frequency of the Cyanophyceae in the locality in question, and this primitive method cannot be absolutely rejected, even though it is undoubtedly subject to numerous sources of error. MOORE and KARRER (1919), too, thought that they could draw certain conclusions from enrichment cultures as to the numbers of algal germs present in the soil. They had the idea that the more luxuriant the algal vegetation was in the cultures, the more germs there were in the soil. This, however, is so uncertain that it is hardly possible to attribute any value to these conclusions. GISTL (1932, 1933) set up several enrichment cultures from each sample of soil, with various media, and has thus made more species of algae grow than usual; from a single locality he found 43 species in all. Through these cultures he tried to elucidate the dependency of the algal vegetation on the condition of the soil as to manure, and on its content of various anions and cations. He does not, however, merely use the number of species found in the soil as an expression of its quality as a substratum for algae. He would also draw comparative quantitative conclusions from the enrichment cultures as to the numbers of germs present in the various soils. He has e. g. established parallel cultures of samples of equal size of two different soils. In one the algae develop rapidly and form a vigorous growth; in the other more slowly. At a suitable time the cubic content of the algal growths in the two cultures is determined, and he then thinks that the mass of algae developed within the same space of time must be a relative measure of the number of algal germs contained in the two soil samples. Such a method is very uncertain. It can only be used if the algal flora contained in the two samples to be compared consists of the same species; it is a common observation that different algae grow at very unequal rates on the same substratum. If, then, the alga flora is not uniform in composition, no certain inferences can be drawn by this method as to the proportional germ content of the two soils. Besides, the numerical proportion of the algal mass of two enrichment cultures will undoubtedly change with time. Further, the competition between the species in the cultures will be able to alter the picture completely. Even if we entirely disregard this attempt to compare the quantity of algae in the soil, GISTL's investigations nevertheless give a fairly distinct picture of the relationship between fully manured soil, deficiently manured soil, and unmanured soil, when the number of species is the only factor considered.

He finds in

Fully manured soil	43 species
Soil manured with kainite and superphosphate	27 —
— — — superphosphate and ammonium sulphate	20 —
— — — kainite and ammonium sulphate	17 —
Unmanured soil	9 —

He further investigates the effect of various anions on the algae of a soil. The anions examined by him, viz. SCN, Cl, SO_4, PO_4, showed a somewhat different effect on the algae. In high concentrations they all check the growth of the algal vegetation, but in somewhat different degree. SCN checks the growth most, PO_4 least. Only in a $^1/_{100}$ mol. solution of the salts in question do the greatest possible number of algal species develop.

If we are to sum up briefly what has been achieved by means of enrichment cultures, we arrive at the result that they have served to deepen the picture of the algal flora obtained by floristic-ecological research. We now know that algae, Chlorophyceae, Cyanophyceae, and Diatomeae, are found everywhere on the surface of the soil, on cultivated land as well as on uncultivated natural soil. According to the information at hand, the number of species is greater on cultivated manured soil than on uncultivated soil, especially when the latter is covered with other vegetation. Damp soil is richer in species than dry soil. Of the numbers of individuals this method merely gives uncertain information. As has already been stated, the presence of the subterranean algae has also been discovered by enrichment cultures. This will be discussed at greater length in a later chapter. Finally, it has been possible, by means of enrichment cultures, especially in connection with observations in hanging drops, to follow the life cycle of individual species (BOYE PETERSEN 1932 I).

b. Dilution Cultures.

The density of species, i. e. the number of species occurring in 1 c.c. of a given soil, undoubtedly gives an impression of the quality of the soil as a substratum for algae, and for many studies this will be an adequate basis. If, on the other hand, the botanist begins to consider the importance of the algae for the biochemical processes in the soil and their interaction with other organisms, he would like to know something more, viz. how many individuals are present in the soil. As has already been stated, MOORE and KARRER (1919) and ESMARCH (1914) therefore tried to form an estimate of the number of algae in the soil; but they only arrived at a relative estimate, at any rate not at definite numbers. MOORE and CARTER (1926) tried to estimate the frequency of the algae in the soil by using inocula of various volumes (10—0.1 gr) for the cultures. The method is very imperfect, and they did not use it to any great extent.

The first to adopt a suitable method for determining the numbers of the algae in the soil was BRISTOL ROACH (1927 I, p. 565; 1928 I, p. 747). Her method is briefly as follows:

10 gr of soil are shaken with 100 c.c. of sterile nutrient medium (inorganic salts) for half an hour. 50 c.c. of this soil suspension are added to 50 c.c. of the same nutrient medium. Of the mixture 50 c.c. are again added to 50 c.c. of fresh nutrient medium and thus she continues until she has in all 17 soil suspensions of the

dilution degrees $^1/_{10}$—$^1/_{655\cdot360}$. Of each of the suspensions 3 cultures are set up in test tubes each containing 15 c.c. of purified sand, to which are added 5 c.c. of the suspension in question. Thus 51 test-tube cultures are obtained which are placed in the light. In a greater or less portion of the tubes algae now develop, and from the number of tubes in which no algae appear the number of germs found in the soils is then calculated.

The method is especially suitable for Diatomeae and partly also for Chlorophyceae, since the cells of these algae can be separated more or less completely from each other by shaking. For the Cyanophyceae, on the other hand, the method is very unreliable, for the tough mucous sheaths of these algae withstand shaking, so that the cells do not separate. BRISTOL ROACH has further, on a previous occasion (1926, p. 152), pointed out another error attaching to the method. If one employs a simple mineral salts solution without any admixture of organic substances, autotrophic algae will develop, but none of the saprophytic ones.

By means of this method BRISTOL ROACH (1927 I, p. 571) at the surface of a manured field found altogether 61,893 alga germs per gr of soil. Further it turned out that the number fluctuated very much in the course of the year. In another locality with manured soil she found in April 105,550 germs, in November only 91 per gr of soil. The difference between the numbers of germs on manured and on unmanured soil was especially marked in April, less so at other seasons. She also found the number of germs of individual species by this method and these are perhaps the most reliable results.

BRISTOL ROACH's method has also been adopted by FEHÉR (1933, p. 22) for the determination of the numbers of algae in forest soils from various parts of Europe. He, too, finds (l. c., p. 175) that there is a seasonal variation of the numbers, but unlike BRISTOL ROACH, he finds that the total is higher in November-December than in July-August; on the other hand, the different groups behave differently according as they come from coniferous or dicotylous woods, and according to the locality. Thus he finds that in Central Europe the number of Chlorophyceae in coniferous woods is higher in winter than in summer; in dicotylous woods the reverse is the case. In Northern Europe the number in coniferous woods is higher in summer than in winter, in dicotylous woods, the reverse.

RICHTER and ORLOWA (1928) have adopted an essentially different method, devised by WINOGRADSKY (1925 I) for use in studies on soil bacteria. A definite quantity of soil is spread over a surface of silicic acid jelly permeated with a nutrient solution of inorganic salts. By this method they found a great many species of green algae, Diatoms, and blue-green algae, arriving at a number of about 50,000 per gr. of soil. Further they have made experiments which show that the degree of acidity of the medium greatly influences the composition of the algal flora realised, since its individual components require a definite hydrogen ion concentration for optimal growth.

In connection with the dilution cultures I will briefly mention a couple of methods for the direct investigation of the micro-flora and micro-fauna of the soil. They have been devised by CONN (1918), WINOGRADSKY (1925 II) and KOFFMANN (1928, 29, 31), and consist in rendering visible the micro-organisms present by a suspension of the soil in water, and staining on a slide. The procedures are rather complicated and difficult to carry out. I have not adopted them in the present work. CHOLODNY's method (CHOLODNY 1930) consists in placing slides in the soil in a vertical position. After some time they are taken up again and particles of soil and the different micro-organisms of the soil will then adhere to the glass. These may thereupon, perhaps after staining, be observed directly under the microscope, the worker will then receive an impression of the form and mode of growth of their colonies. VERPLANCKE (1932) has further developed this method so that it can also be used for determining the numbers of the micro-organisms. He weighs the slides before introducing them into the soil; and after they have been taken up and there only remains, after washing and drying, the quantity of soil on the glass which is to be directly investigated, he weighs them again. He then knows how much soil adheres to the glass and can calculate the number of organisms per gr of soil. In fully manured soil he found 250 million bacteria per gr of soil, and of algae from 3,700 to 10,000 per gr of soil.

With the different methods thus adopted the number of algae in the soil are generally found to range from about 10,000 to 100,000 per gr of soil. However, the number of soils subjected to quantitative examination up to the present is too small to warrant any further conclusion; but it is probable that the dilution cultures afford a means by which it will be possible to solve a number of questions concerning the algal flora of the soil.

c. Unialgal Cultures.

The value of unialgal cultures as a means of scientific research is estimated very differently by different authors. It cannot be denied, however, that many satisfactory results have been obtained by the help of unialgal cultures, even though much that was thought to have been proved by means of such cultures must nowadays be doubted. Thus in a certain period it was thought that experiments in the physiology of nutrition could be profitably made with such cultures; this is undoubtedly to be condemned, and such experiments should only be made with absolutely pure cultures. On the other hand, unialgal cultures will be excellent for many investigations on the morphology and life-history of the algae and on certain physiological subjects (see for instance WETTSTEIN 1921). This is not the place to enumerate the whole extensive literature published on unialgal cultures (in earlier times often passed off for pure cultures) and the results obtained by them. I may here refer the reader to KUFFERATH (1929), who has a systematic review of the whole literature.

I shall confine myself to mentioning some of the chief works of this kind which deal with soil algae. KLEBS (1896) in his well-known work has furnished important data relating to various soil algae, such as *Hormidium* species, *Bumilleria sicula* and *B. exilis*, to which I shall come back in another section, and GERNECK (1907) by his cultivation experiments obtained material for the description of a series of algae of which not a few are soil algae. His descriptions, and particularly his drawings, are not always very good, which has sometimes made it difficult to identify his species; but this does not exclude the possibility that the method in itself may be useful. As a matter of fact, I have myself used unialgal cultures in some works (BOYE PETERSEN 1915, 1932 II). If the worker employs unialgal cultures from one algal cell, as has been done in recent times, e. g. with lichen gonidia (JAAG 1929), he will be working on a scientific basis even though the cultures do not become free of bacteria, as long as, in using them, he bears in mind that bacteria are present.

d. Pure Cultures.

The literature on pure cultures, in which only one species of alga is present and no other organisms, is overwhelming. It has been reviewed and presented in summary form several times, to which summaries the reader is referred (O. RICHTER 1911, 1913, KUFFERATH 1929).

R. CHODAT (1909, 1913) in particular, has with great force asserted the necessity and great advantages of pure cultures over cultures containing bacteria, and has rendered great services to science by devising methods according to which it is possible to realise such cultures. In the somewhat earlier literature the name pure culture is often used about cultures containing bacteria also, and as a rule it is very difficult to see from the presentation what kind of cultures have been at hand. KUFFERATH (1929) has surveyed the literature systematically in this respect. MIQUEL (1892) (not 1890, as stated by KUFFERATH!) is said to have been the first to realise pure cultures of Diatoms free from bacteria. BEYERINCK (1890) was the first to obtain Chlorophyceae in pure culture. The Cyanophyceae have proved the most difficult to obtain in pure culture. PRINGSHEIM (1913) states that he has realised such cultures. Later on DREWES too (1928), and ALLISON and MORRIS (1930 I, II) obtained some few species of *Anabaena* and *Nostoc* in pure cultures devoid of bacteria. KUFFERATH (1912) realised *Porphyridium cruentum* in pure culture. It is a great difficulty in the study of the literature on the pure cultures of algae that in many cases the authors have given very inadequate information about the algae cultivated. It is stated by an author, for instance, that he has cultivated *Cystococcus humicola* Naeg. If no further information is given as to the appearance of the alga, it is impossible to know what species it was, since no one has as yet been able to recognise NAEGELI's species with certainty. It is now known that different green algae are far from behaving uniformly in a physiological respect, and it is, therefore, impossible to generalise about the green algae from a chance paradigm. Physiological investigations on an alga which has not been determined with accuracy, or of which no sufficiently exact description is given, are as a rule without interest.

If one studies the figures in the available works on pure cultures of algae, one is struck by the fact that the algae under consideration appear in a quantity of abnormal forms which are not met with in nature. This is no doubt caused by the fact that such algae are generally kept on purely artificial substrata, such as mineral salt agar or artificial liquid media. If natural substrata were used more extensively, e. g. sterilised soil or a soil decoction as far as the soil algae are concerned, the result would probably be quite different. Such cultures would surely be preferable for systematic and morphological purposes. WETTSTEIN (1921) has, indeed, made a suggestion in that direction.

It is difficult to enumerate the authors which have isolated and cultivated soil algae in pure cultures, since very often it is not stated whence the cultivated species are derived. Hence I can only mention the most important, and the cases in which it must be regarded as certain that the cultures were really free from bacteria.

BEYERINCK (1893, p. 368), by spreading garden soil on a gelatinous surface with a decoction of the leaves of Papilionaceae, realised *Chlorella vulgaris* and cultivated it on malt extract gelatine; he also mentions Diatoms and a small "sarcine-artige Phycophycee"; but he can hardly have obtained these species in pure culture. GRINTZESCO (1903) has cultivated *Chlorella vulgaris*, CHODAT (1909) various algae that may occur in soil, such as *Heterococcus viridis*, *Stichococcus* species, *Hormidium flaccidum*; but it is not stated clearly whether he realised them all in pure culture. HOFFMANN-GROBÉTY (1912) mentions cultures of *Botrydiopsis minor*. Several of the algae mentioned by CHODAT (1913) are undoubtedly soil algae; but it is not stated expressly about any of them that they were derived from soil. On the other hand, BRISTOL ROACH (1926, 1927 II, 1928 II) and BRISTOL and PAGE (1923) have employed pure cultures of algae isolated from soil for a series of experiments on the physiology of nutrition which I shall have occasion to discuss later on. Other authors, such as BONAR and GOLDSMITH (1925) and F. CHODAT (1928 I and II, 1931), have likewise announced that they have set up pure cultures of soil algae. Some new species have recently been described by KOL and CHODAT (1934) and by DÖNZ (1934).

II. The Subterranean Algal Flora.

The first to notice that there are living algae in the deeper layers of the soil, as well as on the surface, was ESMARCH (1911). It was almost by chance that he made this discovery. He wished to procure information, in an indirect way, about the Cyanophycea flora in the various German colonies. He took it for granted that if he procured samples of soil from the colonies, spores of Cyanophyceae capable of germination would be present in them, and by means of a suitable method it should be possible to make these spores germinate and form fully developed plants which could be determined. He then had 90 samples of colonial soil sent to him, partly surface samples (from a depth of 1—25 cm), partly corresponding samples of the subsoil (from a depth of 25—50 cm), mostly from cultivated areas, but also from uncultivated tracts. The samples were cultivated according to the method described above (p. 14), and he succeeded in making a large number of species develop so well that they could be determined. It then turned out that Cyanophyceae developed from several of the subsoil samples, too. From 71.5 p. c. of the surface samples he obtained algae, but only from 28 p. c. of the samples from the deeper layers. He draws the inference that these latter are in the main poorer in Cyanophycea germs than the former. He further found that the cultivated soil was richer in Cyanophyceae than the uncultivated soil. From 67 p. c. of the samples of cultivated soil he obtained a growth of Cyanophyceae, but only from 27 p. c. of the samples of uncultivated soil. It is, moreover, worth noting that he did not find other species in the subsoil samples than he found in the corresponding surface samples, but fewer; later investigators have arrived at the same result.

ROBBINS (1912), independently of ESMARCH, found living algae in Californian soils. His method of investigation was mentioned above (p. 14). The soil samples examined by him were derived from the upper 3—4 in. thick layers, and most of them from cultivated soil; a single sample only from uncultivated soil. In many of the samples he found algae, especially Cyanophyceae, a single Diatom species and a single Chlorophycea, but only from cultivated soil. No algae developed from uncultivated soil. ROBBINS does not seem to have distinguished clearly between the surface algae and the algae of the deeper layers.

This, on the other hand, was the case with FRANCÉ (1913) who, by quite a different method, viz. by direct microscopical examination of soil samples, observed that there occurred living algae a good way beneath the surface. He investigated 180 localities at depths of 0—2 cm, 2—20 cm, and more than 20 cm, and found that the number of algal species was much higher on the surface of the soil than in the deeper layers. It is not certain, however, that all the Diatoms mentioned in his work were living at the depths indicated; this does not appear clearly from his exposition. He observed that Cyanophyceae as well as the true soil diatoms occur not only on the surface of the soil but also down to depths of 50 cm. He even gives definite numbers of individuals, viz. at a depth of 5 cm, in meadow soil, approximately 300 individuals of Diatomeae in 1 mm^3 of soil, "for the most part living, with perfectly fresh chromatophores." He even goes so far as to set up a stratification of the algal flora of the soil, stating that

a depth of 1—5 cm is characterised by Chlorophyceae.
— - 5—20 - — Diatomeae.
— - 20—50 - — Cyanophyceae.

He compares this with the distribution of the algae in the sea, where green algae grow in the upper layers, and next brown algae, while red algae predominate in deeper water. He also has a vague idea that conditions of illumination are responsible for this stratification of the algal flora in the soil. All this, however, is, as far as can be seen, based on a very scanty material of observations, and does not agree with what other investigators have found. Francé did not know Esmarch's observations from 1911, just as the latter did not know those of Francé when in 1914 he continued his work on the problems arising out of his first researches, by a similar investigation of German soils. He first received corroboration that here, too, Cyanophyceae occurred on the surface of the soil as well as in the deeper layers. He investigated a total of 129 "Tiefenproben" from somewhat different depths (10—c. 50 cm). In arable soil, meadow soil, and damp sandy soil from the banks of the Elbe, the margin of a pond, and the shores of the Baltic he found algae in the samples from the sub-soil, whereas there were none in samples from forest, heath, and bog. Esmarch's results have been tabulated in an easily surveyable form by Russell (1923, p. 102). It should be noted that Esmarch's method of taking samples from the deeper layers (l. c. p. 247) does not quite exclude the possibility of an intermixture of particles from the surface layer; he digs a hole and takes his samples from the side of the hole without further precautions. Thus particles sticking to the spade may have been carried down with it and adhere to the surface of the sample and be mixed with it. MOORE and KARRER (1919) would seem to have been somewhat more careful to exclude the possibility of mixing the layers. They state that the spatula with which the samples were taken was sterilised between each sampling. No details of the method of sampling are given, however. Their culture method is mentioned at p. 15. They examined the soil in the Missouri Botanical Garden to a depth of 1 m, and found living algae right down to this level, viz.

Protoderma viride (according to MOORE and CARTER 1926, p. 113
= *Chlorococcum humicola* (Näg.) Rab.)
Hantzschia amphioxys
Navicula atomoides.

These investigations were repeated and amplified by MOORE and CARTER (1926). Sampling and cultivation were carried out in the same way, and here neither a detailed account is given of the procedure in sample-taking, a factor of the utmost importance when enrichment cultures only are used and not dilution. Samples were taken down to a depth of 10 feet; algae were found down to a depth of 9 feet 5 in.; but from greater depths than about 5 feet 5 in. most of the samples gave no development of algae, despite the fact that a large inoculum was used (more than 5 gr).

In the meanwhile the question of the subterranean algae had been taken up in England and a kind of preliminary report was given by RUSSELL (1923). The more detailed treatises were only published after some years. Two methods were adopted. Partly it was attempted by means of quantitative investigations to form an idea of the amount of algae present in the deeper layers of the soil, and partly by means of physiological investigations on particular species of soil algae to form an estimate as to whether, with their mode of nutrition, there could be any probability of their thriving in the soil. BRISTOL ROACH (1927 I), for the taking of samples from various depths, adopted a method, very accurately described, which seems fairly reliable. Since, moreover, the samples were examined by means of dilution cultures (see p. 18) there is no reason to doubt the correctness of the results. In the main two localities only were investigated, viz. a manured and an unmanured plot from the Broadbalk experimental wheat field at Rothamsted. Samples were taken from the surface, and from depths of 2, 4, 6, and 12 in. Further, samples were taken at different seasons. It then appeared that there were great seasonal fluctuations in the number of algae in the soil, and in the winter especially the number at the surface was often very low. At a depth of 6—12 in. the number was always much lower than at the surface; but at a depth of 2—6 in. it was most frequently higher in the cases investigated. As we shall see later on, it is to be regretted that the number of algae was not in all cases determined at these depths.

The investigations on individual species of soil algae (BRISTOL ROACH 1926, 1927 II, 1928 II) will be discussed later on.

Recently other authors, too, have occupied themselves with the subterranean algal flora, thus BOYE PETERSEN (1932 II), FEHÉR (1933, p. 178), and GISTL (1933, p. 363); they have, however, not added fundamentally new fact to what was already known.

In earlier times the occurrence of chlorophyll or related colouring matter in a plant was regarded as a proof that it required light in order to develop normally. FRANCÉ (1913, p. 42) thinks that, in spite of all, algae below the surface of the soil can live as autophytes and procure the necessary organic substances photosynthetically (cf. p. 25). FEHÉR (1933, p. 178) would appear to hold the same view. It must, however, be allowable to assume that the light can only penetrate some few mm into the soil, and that at somewhat greater depths absolute darkness prevails. This is indeed the opinion of all other authors who have dealt with this question, and they do not think, therefore, that the algae can assimilate any carbonic acid by the aid of the light, but hold that they are either in a state of rest or live as heterophytes on the organic substances present in the soil. Thus ESMARCH (1911, 1914) thinks that the Cyanophyceae in the form of spores have been carried down into the ground by water seepage and by burrowing animals, or by the tilling of the soil. He makes an ingenious experiment, which shows that when the Cyanophyceae come below the surface, the vegetative cells fade after a shorter or longer time, and only spores and heterocysts remain. His procedure was as follows. A sheet of filter paper on which there was a dense growth of algae was placed on the surface of a layer of soil 7—8 mm thick in a deep Petri dish and covered with a layer of soil about 1 cm deep, after which the dish was closed, wrapped up in black paper and placed in a dark cupboard. In 5 series of experiments with several different species the above-mentioned changes took place. He further showed that the remaining spores were still capable of germinating so that when a fresh piece of filter paper was laid over the dish and it was exposed to the light, it was shortly afterwards penetrated by Cyanophyceae and covered with slimy layers of them. MOORE and KARRER (1919) think that the algae are present in a vegetative state and grow in the soil, since it seems incomprehensible to them that algae should be capable of being washed down through compact clay soil by the water filtering through. In the soils examined by them they neither found holes from earthworms or passages for surface organisms. They also attempted to prove experimentally that soil algae can grow in the dark below the surface of the soil (1919, p. 305). These experiments, however, do not seem very convincing (thus it would appear, amongst other things, that light was not entirely excluded in the experiments), I shall not, therefore, describe them at length (see, however, p. 63). MOORE and CARTER (1926) call attention to the fact that several physiologists have found that certain green algae are able to live saprophytically in the dark and even retain their chlorophyll. This, they think, explains how the algae can grow in the soil.

BRISTOL ROACH (1927 I, p. 572), too, holds that the algae grow in the soil. She thinks that the fact shown by her figures, that there are often more algae at a depth of 2—6 in. than at the surface, can only be explained by assuming a growth of the algae in the soil. Unfortunately she chiefly has figures for the algae in the deeper layers from the periods when the number at the surface is lowest. It is not improbable that the surface algae, which are most exposed to climatic changes both in respect of drought and intense insolation, with extremely high temperatures in the summer and strong frosts in the winter, may on that account show very varying numbers, while the numbers of the sub-soil algae will be more constant by reason of the more constant life conditions. I think that in periods when surface algae abound, so many may be carried down under the surface that their number in a succeeding period of unfavourable weather may exceed that of the surface algae, and I cannot see that the observations lend essential support to the assumption that the algae grow in the soil.

But she goes a step further and attempts to show experimentally how many of the algal cells in the soil are in a resting condition and how many in a vegetative condition. Her method is to divide a well mixed soil sample into two equal portions; one of them is used for establishing the number of algal germs in the untreated soil, the other is subjected to intensive desiccation and then the number of algal germs present is determined. As was to be expected, the number is now considerably lower than in the untreated sample, and she then takes it for granted that the cells which have resisted the rapid desiccation must have been in the resting state, while those that died were in the vegetative state. The different algae behaved differently in this experiment.

Diatoms were all killed off.

Cyanophyceae seemed in the main unaffected.

"*Pleurococcus vulgaris* Men."	33 p. c. of the cells survived
Chlorococcum humicola	8.5 — - — — —
Chlorella sp. *Heterococcus viridis*	1 — - — — —
Bumilleria exilis..................	quite few cells survived.

All in all only somewhat over 10 p. c. of the algae survived the desiccation process. There is hardly any reason to doubt the correctness of the result. But it gives no information as to what it was desired to learn, viz. whether the algae in the soil are able to live saprophytically and grow within the soil. For there is nothing to hinder the cells from being in a vegetative state, though not growing on that account; their vital functions may be reduced to a minimum owing to the lack of food but they need not for that reason be protected in any way against drought.

The second series of investigations at Rothamsted were likewise carried out by Bristol Roach (1926, 1927 II, 1928 II). They are admirably executed experiments with pure cultures of various soil algae by which it is shown that they are able to grow in the dark when a suitable organic substance is present. The individual species respond differently to the different substances. It must be pointed out, however, that hardly any of the organic substances employed are of common occurrence in the soil, hence it cannot be inferred with certainty from these experiments that the algae in the soil are also able to grow in darkness within the soil. (Boye Petersen 1932 III, p. 15).

If a brief summary is to be given of what these investigations have taught us about the subterranean algal flora, it will be the following facts.

Algae are present in the soil below the surface down to a depth of 2 m, in cultivated as well as in uncultivated soils. There are always most species on the surface, and the number of species decreases rapidly with increasing depth. No species have been found in the deeper layers other than those also present in the corresponding surface layer. The number of algal germs found is sometimes greatest at the surface, but sometimes at the 4—6 in. depth. At greater depths the number of germs diminishes rapidly. Within the soil there occur Diatoms, Cyanophyceae, and Chlorophyceae, and probably also Flagellates. Within the soil the algae are in great part in a vegetative state, but it cannot be stated with certainty whether they are able to grow below the surface.

III. The Occurrence of Subterranean Algae in Denmark.

A. Methods.

1. Sampling.

A very important matter in the study of subterranean algae is the devising of a reliable method for taking up absolutely unmixed samples of soil from various depths. ESMARCH (1911, 1914), MOORE and KARRER (1919), and MOORE and CARTER (1926), the first investigators, do not seem to have worked with sufficient care. The best method so far has been devised by H. M. MORRIS for the study of protozoa, and has been adopted and described in more detail by BRISTOL ROACH (1927 I, p. 567, 1928 I, p. 745). Four iron plates were driven into the ground at right angles to one another so as to enclose a block of soil 9 in. square. A hole was dug on one side of the enclosed block and the plate thus exposed was removed. By means of a flat-bottomed scoop the different layers of soil could now be removed in succession without any risk of mixing. After a layer of soil had been exposed a sample could be removed from the exposed surface which had first been cleansed with a sterilised knife.

The method adopted by me is somewhat simplified. I employ three steel plates, about 2 mm thick, 40 cm long, 10 cm broad. The plates are furnished with a cutting edge below and with a wooden handle at the top. They are made slightly convex in order to increase their stiffness. They are driven into the ground so as to enclose a prismatic column of soil with convex sides. In arable soil, dune sand, heath, etc. the plates are as a rule easily driven down to their full depth. In forest soil stout roots or large stones may cause difficulties; but after some searching a place can generally be found where they can be driven down. The earth is dug away outside two of the plates and the plates are removed. A column of soil is then left whose two somewhat convex sides are exposed, and as a rule the soil will be so firm that there will be no mixing of the layers when the plates are removed. The samples are now taken from the sides of the column at the depths desired. To prevent particles of soil, carried down with the plates, when driven in, from being included in the samples, that part of the column where the samples are to be taken is first cut clean with a strongly heated or preferably a red-hot knife. For heating the knife a small soldering lamp was used. The samples were taken from the side of the column of soil by driving the mouth of a sterile tube into the wall of earth in the purified place, until it was filled with a suitable quantity of soil (c. 5 c.c.). This method of sample-taking seems to me safer than the methods by which the soil is scooped out into the tube with a scoop, and I think I have succeeded in obtaining quite unmixed samples of soil at the various depths.

But even if it could be supposed that in spite of every precaution small particles from other layers of soil had nevertheless made their way into the samples, this will presumably have been unable to influence the results appreciably, since all the samples have been examined quantitatively by means of dilution cultures.

2. Methods of Dilution.

The dilution method adopted by me is a simplified form of the one devised by Bristol Roach (1927 I). The method of the latter investigator necessitates the use of so large a number of tubes that it will be very difficult to make a microscopical examination of the contents of all these tubes. I have, therefore, preferred to dilute to $^1/_{10}$ each time instead of to one-half. The details of the procedure have been previously described (1932 II, p. 17). Bristol Roach (1927 I and 1928 I, p. 747), too, has pointed out the drawbacks of such a method of dilution in the case of soil algae. For Diatoms and many unicellular Chlorophyceae it gives reliable results; but for a good many filamentous Chlorophyceae and especially for Cyanophyceae it is impossible to obtain reliable results since even the most vigorous shaking will not break up these algae into separate cells. Bristol Roach (1927 I, p. 566), be it said, tested her method by diluting equal portions (10 gr) of a well mixed soil sample separately and obtained satisfactory, identical results.

A very important question in such a method is how long and how vigorously the soil sample should be shaken with water in order to separate out as many germs as possible. Concerning this, experiments have been made for fungi by BRIERLEY, JEWSON and BRIERLEY (1927, p. 7). Filamentous algae must be supposed to show in all essentials the same response as fungi. It turned out that shaking in the hand for 20 minutes as a rule gave the maximum number of germs; prolonged shaking was even able to diminish the number.

Hence I have elected to shake the soil sample with water for 20 minutes.

In diluting a soil suspension of the type here considered the ordinary pipettes for chemical experiments cannot be used, the lower aperture being too narrow. I have therefore caused pipettes of 1 c.c. to be made, the lower aperture of which was about 2 mm in diameter. The accuracy of the numerical results obtained is of course less with my method than with that of BRISTOL ROACH; in my opinion, however, the degree of exactitude will suffice when only rough comparisons are intended.

In the sequel I have, for each dilution of soil samples taken in nature, collected the results in two tables. The first table states how many species were found in the individual tubes in the liquid dilution, and the total number of germs found in this way and by dilution in agar. As regards the calculation of the number of germs in the liquid dilutions it should be noted that, if in the two tubes with the highest degree of dilution at which there are still algae, there occur more than one species, I take this into account in the calculation, so that e. g. when in Table 11 (p. 42) there were 1 and 3 species in tubes 5a and 5b, the total number of germs is calculated to be 400,000, since there must necessarily have been at least 4 germs in these two tubes. On the other hand, no account has been taken of the number of species in the tubes of lower numbers, since this would not alter the result appreciably and moreover give very uncertain results.

The second table gives the species found in the samples, and states in how many of the tubes in the liquid dilution any particular species was noticed. This will give some idea of the proportional content of the species in the samples. It should be noted, however, that in the first dilutions there will be many species present and the competition between them will be close; therefore the rapidly growing species in these tubes will dominate over the slow-growing ones, which will perhaps be entirely suppressed, or at any rate difficult to detect, when they are present in small numbers in a copious vegetation of other species. *Muriella terrestris*, for instance, could as a rule only be made out in tubes with a strong dilution, whereas it was not possible to discover it in the weaker dilutions. These figures, therefore, cannot be used for calculating the numbers of the individual species in the samples.

In order to test the accuracy of the dilution methods I made some trial dilutions on a different principle to that of BRISTOL ROACH.

I employed unialgal cultures of *Nitzschia Kützingiana* f. *terrestris* and *Pleurochloris magna*. A little of the algal material was suspended in a small volume (about 2 c. c.) of sterile water and shaken well. By means of a Thoma counting chamber it was found how many germs were present per mm³ and a drop or one cubic centimetre of the suspension (i. e. a definite number of algal germs) was added to 5 c. c. of sterile soil. This sample was then at once diluted.

The results will appear from the table below.

Table 1. Trial Dilutions.

	Number of germs sown out	Number found by dilution in liquid	Number found by dilution in agar
I. Nitzschia	675,000	100,000	129,000
II. Pleurochloris	67,000	10,000	66,000
III. Pleurochloris	1,340,000	500,000	840,000
IV. Nitzschia	56,000	10,000	91,000

As will be seen, the dilutions have in general given somewhat lower figures than they should according to the counting in the counting chamber. This, however, is quite natural since some germs must always be expected to sink to the bottom before they can be sucked up by the pipette, or sink to the bottom of the latter during the regulation of the volume of liquid; and they will, accordingly, run out of it in too great amount together with the superfluous liquid. Possibly a number of the germs in the starting material for dilution No. 1 were not really capable of division; the material for this dilution was taken from a rather old culture in which there were already not a few empty frustules.

In contrast herewith the material for dilution No. 4 was taken from a young agar culture in vigorous growth, and small particles of agar were found in the suspension, enclosing numerous Diatom cells which had penetrated into the agar. These agar clumps were then presumably split up at the shaking of the soil sample and their germs liberated, which explains that the dilution could give higher figures than resulted from the counting in the counting chamber.

In all the trials the agar dilutions gave the best results. Hence in this work for each dilution in liquid I have as a rule also set up dilutions with agar (cf. BOYE PETERSEN 1932 III, p. 17) of 1 c. c. of dilution No. 1 and frequently also of No. 2, and thence obtained an often very significant corrective for the liquid dilution.

In certain cases, when germs occurred in special abundance, I was also by direct counting of these able to verify the results of the dilution method; on this subject the reader is referred to the tables in the sequel.

The counting of colonies in an agar dilution often presents certain difficulties in the case of a mixture of algal species as obtained in dilution of a soil sample from nature. In the first place the colonies develop at a very unequal rate of speed, and it is, therefore, difficult to be sure that the smallest colonies have been included. On the other hand, the time in which an agar dilution can be left to stand is limited by the fact that the agar dries up; the suitable time for making the count must therefore be chosen as judiciously as possible. Another difficulty lies in the fact that a number of Chlorophyceae form zoospores which spread in the condensing water and form new colonies, and it may then sometimes be difficult to decide whether a colony is of primary or of secondary origin. Diatomeae and Cyanophyceae will move away with great briskness from the centre of the colony, and the latter will become rather blurred. None the less, there can as a rule be no doubt as to where the starting point of the colony is situated. Occasionally a vigorous development of fungi destroyed some of the agar dilutions.

If the number of colonies was small, I simply counted them directly, sometimes after marking the position of the individual colony with a circle of ink under a magnifying glass. If there is a large number of colonies, it is impracticable to count them all, and I have then tried using an ordinary WOLFHÜGEL's counting plate. Since, however, the colonies can only be seen against a white background, I had to give up this method and instead I used a piece of squared paper in which a square larger than the Petri dish was subdivided by heavy pencil lines into square centimetres. 10 random squares were numbered 1 to 10, and the colonies within the squares in question were counted, I could then by a simple calculation find the approximate number present in the dish.

B. Preliminary Investigations of a single Locality in the Botanical Garden, Copenhagen.

1) As a first experiment I collected a series of soil samples down to a depth of 30 cm in the Botanical Garden. These samples were diluted according to the method of BRISTOL in a total of 17 dilutions, each being diluted to one-half, and from each dilution three 5 c. c.-cultures were set up in test tubes, i. e. 51 cultures in all.

According to FISCHER (ABDERHALDEN's Handbuch Abt. XI, Teil 3, p. 750, BRISTOL ROACH 1927 I, p. 565) the numbers of algal germs present were then calculated from the numbers of blank cultures in each series.

As regards the locality the following particulars can be given:

The samples were taken in deep shade from a couple of horse chestnuts (*Æsculus* sp.) where the soil is not dug up, but where there is a more or less dense carpet of *Eranthis hiemalis* in the spring. When the earth was removed from around the iron plates several old *Eranthis* bulbs came to light, which shows that the soil can hardly have been disturbed here for the last 10 years.

Down to a depth of about 20 cm the soil was good black garden mould (pH 7.79), at about 30 cm, red clay (pH 8.06).

Table 2 (p. 36) shows the results of the dilutions.

The number of algae is greatest at the surface, more than 200,000 per c. c. of soil; at a depth of 3—10 cm the number seems to be almost constantly 7—8000 per c. c. of soil, but after this there is a rapid drop, so that at a depth of 30 cm there only occur about 800 per cubic centimetre.

Table 2. Botanical Garden $^9/_4$ 30.
× = algae developed; 0 = no algae.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Germs per c. c. of soil
Surface........	a	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	..	200,000
	b	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	
	c	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	..	
5 cm Depth......	a	×	×	×	×	×	×	×	×	×	×	×	×	×	×	0	0	0	0	6,780
	b	×	×	×	×	×	×	×	×	×	×	×	×	0	0	0	0	0	..	
	c	×	×	×	×	×	×	×	×	×	×	×	×	0	0	×	0	0	..	
10 - -	a	..	..	..	×	×	×	×	×	×	×	0	×	×	×	×	×	0	..	8,626
	b	×	×	×	×	×	×	×	×	×	0	×	×	×	×	0	0	0	0	
	c	..	..	..	×	×	×	×	×	×	0	×	×	×	0	0	0	0	..	
20 - -	a	..	..	..	×	×	×	×	×	×	×	×	0	0	0	×	0	0	..	2,619
	b	×	×	×	×	×	×	×	×	×	×	0	0	×	×	0	0	0	0	
	c	..	..	..	×	×	×	×	×	×	×	0	0	×	0	0	0	0	..	
30 - -	a	..	..	..	×	×	×	×	×	0	×	0	0	×	0	0	0	0	..	817
	b	×	×	×	×	×	×	×	×	×	×	0	0	0	0	0	0	0	0	
	c	..	..	..	×	×	×	×	×	×	×	0	0	0	0	0	0	0	..	

2) Later on ($^{19}/_{10}$ 32) I collected a fresh series of samples from the same locality, diluting them according to my simplified method to $^1/_{10}$ each time and with corresponding dilutions on agar. The results are recorded in Tables 3 and 4.

Table 3. Botanical Garden $^{19}/_{10}$ 32. The figures denote the number of species in each tube.

	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7 b	Nos. of germs per c.c. of soil with dilution in liquid	agar
Surface	6	3	2	4	1	2	1	1	0	0	0	0	0	0	20,000	4,800
10 cm Depth ...	4	4	4	4	1	1	1	0	0	0	0	0	0	0	10,000	4,400
20 - — ...	3	6	3	1	2	1	1	0	1	0	0	0	0	0	20,000	4,300
30 - ...	4	3	3	2	1	1	0	0	0	0	0	0	0	0	2.000	1,900

For the surface layer this dilution gave considerably lower figures than that dating from $^9/_4$ 30, and the number of algal germs proved to be about the same from the surface down to a depth of 20 cm, while the numbers for a depth of 30 cm were considerably

Table 4. Botanical Garden $^{19}/_{10}$ 32. Number of tubes in which each species occurred.

	Surface	10 cm	20 cm	30 cm
Navicula Atomus	5	4	2	2
Pinnularia sp.	2	0	0	0
Bumilleria exilis	1	0	1	2
Chlamydomonas sp.	1	1	3	2
Chlorococcum humicola	2	3	3	1
Fernandinella alpina	2	0	0	0
Heterococcus viridis	3	4	4	3
Macrochloris dissecta	0	0	2	1
Muriella terrestris	4	6	3	2
No. of species	8	5	7	7

lower. Further it was established what species of algae occurred in the various tubes in the liquid dilution. Altogether 9 determinable species occurred, with one exception all found at the surface. There were, however, almost just as many species in the deeper layers as at the surface. The small number of germs found at the surface may be supposed to be due to the season, since the growth of algae is undoubtedly liveliest in the spring when the earth is damp and the illumination good, before the trees come into leaf, while probably a great many algae, especially Diatoms, die off in the summer. As already stated, Bristol Roach (1927 I, p. 572) found that the amount of algal germs at the surface of the soil fluctuates very considerably in the course of the year, and she observed a minimum precisely in the autumn. That the algal flora in this locality is in reality much richer than appears from this dilution, was evidenced by the drought samples mentioned in the sequel, which were collected in the same locality; in these more than 30 species were found.

In Table 3 one is struck by the fact that the agar dilutions as a rule exhibit lower figures than the liquid dilutions. This is no doubt because the agar plates dried up before the algal colonies were able to develop properly, a result of the poor illumination at this season of the year.

3) In order to gain some idea of the effect of my simplified dilution method compared to Bristol's more elaborate one, and further to establish what species can develop in the cultures in

spring I collected a fresh series of samples in the same locality on $^5/_4$ 34. At a depth of about 20 cm I encountered roots of trees 2 cm thick, which had 9 annual rings. It may then be considered as settled that this spot has not been dug up for about 10 years. The results of the dilutions are shown in Tables 5 and 6. At the surface the greatest number of germs was found, which has so far been observed in any dilution, viz. 3,000,000 per c. c. in the liquid dilution (in the agar dilution the number was 1,108,000 per c. c.

Table 5. The Botanical Garden $^5/_4$ 34. Number of species in each tube in the liquid dilution.

	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7 b	No. of germs with dilution in liquid	agar
Surface	13	9	4	5	6	7	3	3	2	2	1	2	0	0	3,000,000	1,108,000
10 cm Depth	3	4	2	3	1	1	1	1	0	0	0	0	0	0	20,000	25,000
20 - —	4	3	4	3	3	1	1	0	0	0	0	0	0	0	10,000	22,000
30 - —	5	5	4	4	1	1	1	1	0	0	0	0	0	0	20,000	13,000

Table 6. Botanical Garden $^5/_4$ 34. No. of tubes in which each species occurred.

	Surface	10 cm D.	20 cm D.	30 cm D.
Hantzschia amphioxys.................	4	0	0	0
Navicula Atomus.......................	8	2	0	1
— Borrichii.....................	2	0	0	0
Pinnularia obscura	6	0	0	0
Bumilleria exilis........................	2	0	0	0
Bumilleriopsis brevis....................	2	0	1	0
Chlamydomonas terrestris..............	2	0	0	2
— sp.	0	0	2	2
Chlorella vulgaris......................	5	0	0	0
Chlorococcum humicola	4	3	4	4
Dictyococcus minor.....................	0	0	0	1
Dictyosphærium minutum..............	0	0	0	1
Fernandinella alpina....................	2	0	0	0
Heterococcus viridis	5	2	4	4
Hormidium flaccidum..................	0	0	0	1
Macrochloris dissecta	0	2	0	0
Muriella terrestris	4	6	6	6
Stichococcus sp.........................	10	0	0	0
Total of species...	13	5	5	9

of soil). At a depth of 10—30 cm the number of germs proved far smaller, viz. ranging about 10,000—20,000 per c. c. of soil. These figures on the whole agree very well with what was found at the first dilution according to the method of BRISTOL. This also lends support to the supposition that the number of germs at the surface is greatest in the spring time, while it is considerably less in the deeper layers, and almost constant all the year round.

In counting up the species that had developed in the tubes, a greater number was found here than in the autumn of 1932, viz. 18 species (9 in October 1932). No blue-green algae did develop.

C. Investigations on some characteristic Danish Soils.

In order to gain some insight into the distribution of the subterranean algal flora in Denmark I collected samples from 7 localities with very heterogeneous soils. For purposes of comparison these samples were all collected in the spring, in March-April, and were all treated according to the same method of liquid dilution. For the last ones agar dilutions were also established.

a. Cultivated soil.

As examples of cultivated soils in which the tilling of the soil must be counted as a factor contributing greatly to convey algae from the surface into the soil, I selected

1. Pasture land on Holmsland near Ringkjøbing.
2. Very poor arable land on Holmslands Klit.
3. Garden soil, Amager.

In the two first localities series of samples were taken in April 1930, and they were diluted according to the simplified method, though only to a dilution of $^1/_{100,000}$. This, however, proved barely sufficient for these localities, so that in the samples from the upper layers one must consider the possibility of a larger content of germs than shown by the dilutions. In order also to obtain an example of the best possible soil I collected samples in my garden at Amager, where the layer of mould is very thick (80 cm) and where the soil has been kept in the best possible condition by means of farmyard manure and artificial fertiliser ("Nitrophoska"). These samples were diluted in liquid dilutions up to $^{1}/_{100,000,000}$ and with corresponding agar dilutions of 1 c. c. of dilutions No. 1 and No. 2.

1. Pasture land on Holmsland near Ringkjøbing $^{14}/_{4}$ 1930. 3-year-old pasture. Clay mixed with sand; 0—25 cm brownish mould, 25—30 cm bluish black sandy clay. 0—5 cm pH = 6.69; 25—30 cm pH = 5.41. Vegetation: grass and clover.

Table 7. Pasture on Holmsland $^{14}/_{4}$ 1930. Number of species in each tube.

	0 a	0 b	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	Germs per cc. of soil
Surface	9	6	4	3	5	6	2	2	3	1	40,000
5 cm Depth	1	1	1	1	3	3	2	2	1	0	10,000
10 - —	2	2	1	1	1	2	1	1	0	0	2,000
20 - —	2	1	1	2	2	2	0	0	0	0	400
30 - —	1	1	1	1	1	0	0	0	0	0	100

Table 8. Holmsland pasture. Number of tubes in the dilution in which each species occurred. Note that the dilutions were made in 10 tubes. Degree of dilution $^{1}/_{10}$—$^{1}/_{100,000}$.

	Surface	5 cm D.	10 cm D.	20 cm D.	30 cm D.
Anabaena sp.	0	1	1	2	0
Cylindrospermum sp.	0	0	2	0	0
Microcoleus vaginatus?	1	0	0	0	0
Hantzschia amphioxys	4	2	0	0	0
Navicula Atomus	2	0	0	0	0
Nitzschia Kützingiana f.	0	0	1	1	0
Pinnularia borealis	2	0	0	0	0
— intermedia	1	0	0	0	0
Bumilleria exilis	10	2	0	0	0
Bumilleriopsis brevis	4	1	0	0	0
Chlamydomonas sp.	2	2	1	0	0
Chlorella vulgaris var.	3	0	0	0	0
Chlorococcum humicola	4	0	1	0	0
Dictyococcus minor	5	3	1	0	0
Hormidium flaccidum	1	0	0	0	0
Macrochloris dissecta	2	0	0	0	0
Pleurochloris magna	0	0	2	0	4
Total No. of species	13	6	7	2	1

Upon dilution 40,000 algal germs per c. c. were found at the surface and the number proved to decrease rapidly downwards. In the surface layer 13 species in all developed in the cultures, and the number of species, too, diminished rapidly downwards. Nearly all the species occurred in the surface layer; some few merely, especially *Nitzschia Kützingiana* f. *terrestris* and *Pleurochloris magna*, were only observed at a depth of 10 cm and more.

The commonest species was *Bumilleria exilis*, which occurred in all the tubes from the surface, i. e. in a number of 10.000 per cubic centimetre.

2. Arable Land on Holmslands Klit near Nr. Lyngvig; $^{14}/_{4}$ 1930. In 1929 spring-sown grain; in 1930 the field had not been ploughed. Vegetation: *Trifolium repens*, *Rumex Acetosella*, *Hypochoeris radicata*, *Alchimilla arvensis*, *Gramineae*. Soil: very sandy. 0—5 cm pH = 5.18; 5—10 cm pH = 5.20.

Table 9. Arable Land on Holmslands Klit. Number of species in each tube.

	0 a	0 b	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	Germs per c.c.
Surface	3	2	3	4	3	4	3	2	0	1	10,000
5 cm Depth	4	4	3	1	3	1	1	1	0	1	10,000
10 - —	3	2	1	2	2	3	1	1	0	1	10,000
20 - —	1	2	2	1	1	1	1	1	0	0	2,000
30 - —	2	2	2	2	1	1	0	0	0	0	200

Table 10. Arable Land on Holmslands Klit. Number of tubes in the dilution in which each species occurred. Degree of dilution $^{1}/_{10}$—$^{1}/_{100,000}$.

	Surface	5 cm D.	10 cm D.	20 cm D.	30 cm D.
Nostoc sp.	0	1	1	0	0
Characiopsis Heeringiana	0	2	0	0	0
Chlorella botryoides	4	7	5	0	0
Chlorococcum humicola	6	3	3	2	0
Dictyococcus irregularis	0	1	0	0	0
— minor	0	0	1	1	1
Hormidium flaccidum	5	0	0	0	0
Monodus subterraneus	0	0	1	0	0
Muriella terrestris	0	0	2	5	6
Pleurochloris magna	7	1	2	0	4
No. of species	4	6	7	3	3

Tables 9 and 10 show that from the surface down to a depth of 10 cm there occurred an equal amount of algal germs; after this the amount of germs rapidly decreased downwards. A total of 10 species was found in the dilutions; the greatest number (7) appeared at a depth of 10 cm; but no great importance can be attached to this fact, since this may easily be due to competition and other more fortuitous circumstances.

Diatoms did not develop in the cultures.

3. Amager; garden soil $^{12}/_{4}$ 34.

Old manured cultivated soil. The layer of mould attains a thickness of 80 cm.
Surface pH = 7.68.
30 cm depth pH = 7.70.

The soil has been dug annually, though in the place where the sample was taken not later than the spring of 1933. The upper layer is somewhat crusty, ash-dry. To a depth of 30 cm the soil was loose and offered no resistance to the iron plates. The sample taken from 30 cm was not quite certainly unmixed. Upon microscopical examination of the 5 c. c. of soil from the surface sample suspended in 50 c. c. of culture medium, numerous Hormidium filaments, round cells of green algae, and Diatoms could be observed, whereas no Cyanophyceae were seen. In the sample from 10 cm similarly examined no algae were observed. The numbers of germs at the surface must at the outset be presumed to amount to hundreds of thousands, while at a depth of 10 cm it would prove much lower. The dilution showed this to be true. Tables 11 and 12 exhibit the results.

Table 11. Garden soil, Amager $^{12}/_{4}$ 34. Number of species in each tube.

	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7b	No. of germs per c.c. of soil found by dilution in	
															liquid	agar
Surface	18	18	13	13	5	6	3	3	1	3	0	0	0	0	400,000	462,000
10 cm Depth	6	5	6	5	1	1	0	2	0	0	0	0	0	0	20,000	8,000
20 - —	5	4	3	4	1	2	0	0	1	0	0	0	0	0	10,000	4,000
30 - —	6	4	2	3	1	1	0	0	0	0	0	0	0	0	2,000	3,200

Table 12. Garden soil; Amager. Number of tubes in which each species occurred.

	Surface	10 cm D.	20 cm D.	30 cm D.
Oscillatoria	3	0	0	0
Phormidium autumnale	3	0	0	0
Achnanthes coarctata	1	0	0	0
Hantzschia amphioxys	8	5	3	1
Navicula Atomus	8	4	6	4
— Borrichii	3	0	0	0
— gibbula var. terrestris	3	1	0	0
— mutica f. Cohnii	4	3	0	0
— — f. Goeppertiana	2	0	0	0
— — f. quinquenodis	3	0	0	0
— — f. minima	3	1	0	0
— thermicola	3	0	0	0
Nitzschia Kützingiana f. terrestris	0	1	3	0
Pinnularia borealis	4	0	0	0
Bumilleria exilis	6	2	0	3
Bumilleriopsis brevis	4	0	0	0
Chlamydomonas terrestris	2	2	3	2
— sp.	4	0	0	0
Chlorella vulgaris	5	1	0	0
Chlorococcum humicola	6	4	3	4
Dictyococcus minor	1	0	0	0
Fernandinella alpina	2	0	0	0
Heterococcus viridis	4	0	1	1
Hormidium flaccidum	2	0	0	0
Macrochloris dissecta	1	0	0	0
Muriella terrestris	0	2	1	0
Total No. of species	24	11	7	6

At the surface more than 400,000 germs of all three groups of algae were found per cubic centimetre of soil. Already at a depth of 10 cm the numbers of germs proved much lower (20,000 per c. c. of soil), and decreased steadily downwards.

A total of 26 species was found, all of which except 2 occurred in dilutions of the surface sample. *Nitzschia Kützingiana* f. *terrestris* and *Muriella terrestris* were only observed at a depth of 10—20 cm.

b. Uncultivated soil.

In order to obtain some information as to whether algae occur, in uncultivated soil too, in the deeper-lying layers, I collected series of samples in the following localities:

1. A dune depression on Holmslands Klit.
2. Borris Heath.
3. Forest ground, Ermelunden.
4. Common, Ermelund plain.

It must be taken for granted that in all these localities the soil has been left undisturbed for a very long time; at any rate the ground has not been broken up within a measurable space of time. It must, therefore, be assumed that the algae have not been carried down into the soil by any human agency, but if they are found in the deeper layers this must be due to normally acting forces of nature.

1. Dune depression on Holmslands Klit near Nr. Lyngvig. $^{14}/_4$ 1930. Vegetation: A dense growth of *Erica*, *Calluna*, *Oxycoccus* and under these a close covering of *Hypnum*.

Soil: 0— 5 cm fairly pure sand (fresh blown sand).
5—15 - black raw humus; pH = 4.83.
15—25 - reddish sand; pH = 5.11.
25—26 - slight formation of hard pan.
26—30 - sand.

Table 13. Holmslands Klit. Depression. Dilutions $^1/_{10}$—$^1/_{100,000}$. Number of species in each tube.

	0 a	0 b	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	Germs per c.c.
Surface	2	1	0	0	1	1	0	0	0	0	200 (2,000?)
5 cm Depth	1	1	0	0	0	0	0	0	0	0	2
15 - —	0	0	0	0	0	0	0	0	0	0	0
20 - —	0	0	0	0	0	0	0	0	0	0	0

7 species of algae in all occurred in the cultures, and algae only appeared in the dilution from the surface and in that from a depth of 5 cm. The surface yielded 200 germs per c. c. But there

Table 14. Dune depression; Holmslands Klit. Number of tubes in which each species occurred.

	Surface	5 cm D.	15 cm D.	20 cm D.
Cylindrospermum sp.	1	0	0	0
Hantzschia amphioxys	1	0	0	0
Chlamydomonas fusiformis	1	0	0	0
Chlorococcum humicola	0	1	0	0
Dictyococcus irregularis	1	0	0	0
Macrochloris dissecta	1	0	0	0
Pleurochloris magna	0	1	0	0
Total number of species ...	5	2	0	0

is a great probability that algal germs have also been present in tubes 1a and 1b, but that for some reason or other they have not developed. If this is taken for granted, we arrive at 2000 germs per c. c. at the surface, rather a low figure, which, however, may be supposed to correspond very well with the extremely poor soil.

2. Borris Heath $^{15}/_{4}$ 1930. Vegetation: *Calluna*, *Cladonia* and other lichens; on the ground mosses and liverworts.

Soil: 0— 5 cm heath raw humus; pH = 3.97.
5—10 - bleached sand; pH = 4.49.
At a depth of 15 cm hard pan.

Table 15. Borris Heath. Number of species in each tube; dilutions $^{1}/_{10}$—$^{1}/_{100,000}$.

	0 a	0 b	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	Germs per c.c. of soil
Surface	3	4	2	3	2	2	3	2	0	2	20,000
5 cm Depth	1	0	0	1	1	2	0	0	0	0	300
10 - —	0	0	0	0	0	0	0	0	0	0	0

Table 16. Borris Heath. Number of tubes in which each species occurred.

	Surface	5 cm D.	10 cm D.
Chlorella botryoides	7	0	0
Coccomyxa sp.?	7	4	0
Chlorococcum humicola	2	0	0
Dictyococcus minor	7	1	0
Total No. of species ...	4	2	0

As will appear from tables 15 and 16, only 4 species of algae occurred here, all of them Chlorophyceae. 20,000 germs per c. c. of soil were found at the surface; at a depth of 5 cm only 300, and none at 10 cm.

3. Forest; Ermelunden $^{24}/_{3}$ 33. A series of samples was collected on a slope with southward exposure in a forest of beech-trees.

Vegetation: *Aegopodium podagraria*, *Agropyrum caninum*, *Anemone nemorosa*, *Arenaria trinervia*, *Ficaria verna*, *Gagea lutea*, *Geum rivale*, *Holcus mollis*, *Melica uniflora*, *Mercurialis perennis*, *Ranunculus auricomus*, *Viola silvatica*.

0— 5 cm black mould; pH = 5.00.
5—30 - clay mixed with sand; at a depth of 20 cm pH = 4.41.

At the 25th cm a big earthworm was observed.

In this locality 17 species of algae were shown to be present (Table 18). The samples from the deeper layers yielded no other species than the surface, but a much smaller number. At a depth of 10—30 cm the number of species was much the same, viz. 3—4. The number of germs proved highest at the surface, but not very high, and it decreased quickly downwards. Nor did I from my previous experience (BOYE PETERSEN 1932 I, p. 12) expect to find any very large number of species in the forest ground.

FEHÉR (1933, p. 176, 178) has found a very varying number of algae in forest soil, viz. from 1440 to 793,300 per gr of soil. As a rule he finds the largest number in November-December, a smaller number in July-August (see further above, p. 19).

Table 17. Ermelunden $^{24}/_{3}$ 33. Number of species in each tube.

	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7 b	No. of germs per c.c. of soil with dilution in	
															liquid	agar
Surface	12	8	4	3	2	1	0	1	0	0	0	0	0	0	10,000	4,000
10 cm Depth	4	3	2	1	0	0	0	0	0	0	0	0	0	0	300	2,000
20 - —	4	1	0	1	0	0	0	0	0	0	0	0	0	0	200	200
30 - —	2	1	0	1	0	1	0	0	0	0	0	0	0	0	200	200

4. Common. Ermelund plain $^{24}/_{3}$ 33. A common which, as far as is known, has never been ploughed. The ground is covered

Table 18. Ermelunden $^{24}/_{3}$ 33. Number of tubes in liquid dilution in which each species occurred.

	Surface	10 cm D.	20 cm D.	30 cm D.
Phormidium foveolarum	2	0	0	0
Navicula Atomus	3	0	0	0
— cincta v. Heufleri	1	0	0	0
— thermicola	1	0	0	0
Pinnularia obscura	2	0	0	0
Bumilleria exilis	3	0	0	0
Chlamydomonas terrestris	3	3	2	1
— sp.	1	0	0	0
Chlorella vulgaris	1	0	0	0
Chlorococcum humicola	1	0	0	1
Dictyococcus minor	3	2	2	3
— sp.	1	0	0	0
Dictyosphærium minutum	1	0	0	0
Heterococcus viridis	4	3	1	0
Muriella terrestris	1	0	0	NB.
Pleurochloris sp.	1	0	0	0
Tribonema bombycinum	1	0	1	0
Total No. of species	17	3	4	4

NB. 1 Colony in agar dilution.

with a dense carpet of grass. Cattle and horses graze here in the summer.

Vegetation: *Achillea millefolium*, *Agrostis tenuis*, *Bellis perennis*, *Cerastium caespitosum*, *Ficaria verna*, *Lolium perenne*, *Plantago lanceolata*, *Ranunculus bulbosus*, *Taraxacum* sp.

0—10 cm dark-coloured, stiff clay mixed with mould; pH = 5.45.
10—20 - red clay mixed with sand.
20—30 - yellowish clay; pH = 4.81.
Earthworms were observed at a depth of 10 cm.

The result of the dilutions (Tables 19 and 20) was that the surface yielded an algal flora fairly rich in species, but especially rich in individuals; passing downwards the number of germs decreased rapidly until, at a depth of 30 cm, only 20 were found per c. c. of soil. At the surface 17 species were found in the dilution; but in a Freudenreich flask in which 5 c. c. of surface soil were moistened with 3 c. c. of BRISTOL's liquid medium, the following additional species developed: *Anabaena* sp., *Phormidium tenue*, *Oscillatoria* sp., *Pinnularia borealis*, and *P. subcapitata*.

Two species which were not observed in the dilution cultures from the surface, but were found in the cultures from the 10—20 cm depth, also developed in the above-mentioned culture of soil from the surface. These were *Navicula Atomus* and *Rhaphidonema sempervirens*.

Table 19. Ermelund plain. $^{24}/_{3}$ 33. Number of species in each tube.

	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	7 a	7 b	No. of germs per c. c. of soil with dilution in	
															liquid	agar
Surface (3 c. c. soil)	10	12	7	6	4	3	2	2	0	0	0	0	0	0	66,000	149,000
10 cm Depth	4	6	2	3	2	2	0	0	0	0	0	0	0	0	4,000	17,000
20 - —	4	2	3	2	1	1	0	0	0	0	0	0	0	0	2,000	3,700
30 - — *)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	20	0

*) In tubes 0 a and 0 b, 1 and 3 species respectively developed.

Table 20. Ermelund plain $^{24}/_{3}$ 33. Number of tubes in which each species occurred.

	Surface	10 cm D.	20 cm D.	30 cm D.
Hantzschia amphioxys	3	0	0	0
Navicula Atomus	0	1	1	0
— Vaucheriae	2	1	0	0
Pinnularia molaris f. curta	1	0	0	0
Bumilleria exilis	6	3	0	0
Bumilleriopsis brevis	1	0	0	0
Chlamydomonas sp.	2	0	2	2
Chlorella ellipsoidea	1	0	0	0
— vulgaris	4	3	0	0
Chlorococcum humicola	2	1	2	NB.
Dictyococcus minor	0	1	0	0
Fernandinella alpina	4	2	0	0
Heterococcus viridis	6	5	4	NB.
Hormidium flaccidum	2	0	0	0
Keratococcus bicaudatus	1	0	0	0
Monodus subterraneus	1	0	0	0
Muriella terrestris	2	1	3	0
Rhaphidonema sempervirens	0	1	0	0
Stichococcus bacillaris	4	1	0	0
Tribonema bombycinum	4	0	0	0
Total No. of species	17	11	5	3

NB. Developed in tubes 0 a and 0 b. (Degree of dilution $^{1}/_{10}$).

c. General conclusions.

The results of the liquid dilutions of samples from the deeper layers of the localities investigated which are brought together in Table 21 may be compared e. g. with the corresponding results in Bristol's tables (1927 I, p. 572, table III). It should be noted, however, that Bristol's figures indicate the numbers of algal germs per gram of soil. On weighing 5 c. c. of air-dry soil from pasture land on Holmsland I found that 1 c. c. of dry soil has a weight of 0.9 gr. It follows that Bristol's figures and my figures can simply be used for a rough comparison, since Bristol's weights of soil do not refer to dry soil, but to soil containing some amount of water.

Table 21. Synopsis of the number of algae found by dilution in liquid from the localities investigated.

	Surface	5 cm Depth	10 cm Depth	20 cm Depth	30 cm Depth
Holmsland, pasture $^{14}/_{4}$ 30 clay; pH = 6.69	40,000	10,000	2,000	400	100
Arable, Holmslands Klit $^{14}/_{4}$ 30; sandy soil; pH = 5.18	10,000	10,000	10,000	2,000	200
Amager garden soil $^{12}/_{4}$ 34; good garden mould; pH = 7.68	400,000	—	20,000	10,000	2,000
Botanical Garden $^{9}/_{4}$ 30; Bristol's meth.; good mould; pH = 7.79.	200,000	6,780	8,626	2,619	817
Botanical Garden $^{5}/_{4}$ 34 = preceding locality	3,000,000	—	20,000	10,000	20,000
Dune depression Holmsl. Klit $^{14}/_{4}$ 30; dune sand pH = 4.83	200 (2,000?)	2	0	0	—
Heath, Borris $^{15}/_{4}$ 30; heath raw humus, bleached sand pH = 3.97.	20,000	300	0	—	—
Forest, Ermelunden $^{24}/_{3}$ 33; loose mould, clay mixed with sand, pH = 5.00	10,000	—	300	200	200
Common, Ermelund plain $^{24}/_{3}$ 33, clay mixed with mould, red clay; pH = 5.45	66,000	—	4,000	2,000	20

Bristol Roach found highly differing numbers of algal germs at the surface of the soil, viz. from 105,550 to 91 per gr of soil, while Fehér (1933) found up to 793,300 per gr of soil. Most of my figures lie within the same limits.

Table 21 shows that broadly speaking the luxuriance of the algal vegetation will be dependent on the richness of the soil; neutral soil rich in mould harbours the greatest number of algae, while the soil of the dune and the heath, which is regarded as very poor soil for phanerogams, is also poor in algae. The forest soil, however, forms an exception (Ermelunden), its content of algae being remarkably low.

These investigations on the occurrence of subterranean algae in Denmark would then seem to warrant the following inferences:

1. Subterranean algae are of common occurrence in Danish soils in cultivated as well as uncultivated places.
2. The number of algal germs is in spring greatest at the surface and decreases rapidly with increasing depth; whether the case is otherwise at other seasons of the year cannot be said.
3. The algal species in the deeper layers of the soil are in the main the same as, but fewer than, the species at the surface of the same soil. All the species found in the deeper layers have also been found at the surface, though not always in the same locality.
4. The quality of the soil seems in the main to be decisive for the luxuriance of the algal flora.
5. On the other hand, it does not seem to have any influence on the number of subterranean algae whether or not the ground has been disturbed; more probably the relation is simply that when there are many epiterranean algae, the subterranean algae will also be comparatively numerous. As previously pointed out, however, conditions may perhaps be different at other times of the year.

IV. The Occurrence of Subterranean Algae in absolutely Virgin Soil in Greenland.

For the purpose of obtaining a series of soil samples from the lower layers of absolutely virgin soil I asked Mr. GELTING to collect such a series in East Greenland. He brought back two series from the southernmost point of Eskimonæs (lat. 74°06′ N.).

As to the locality (Fig. 1) GELTING states that the samples were collected in a place where the whole of the vegetation was very luxuriant, amongst other things because just above it there were nesting-places for numerous barnacle geese whose droppings trickled down on to the spot below with the rain water.

The place in question was a slope with a southward exposure, sharply divided into terraces. A rocky wall (gneiss) 15 m high descended from the nesting-place of the barnacle geese, after that came a terrace 10 m wide, then a wall 2 m high, below which the samples were taken on a plateau 2—3 m wide (by the arrow in Fig. 1). The snow remains on the terraces all through the winter, so that these places must be called snow patches. The surface of the ground is covered with a dense layer of moss and with a growth of *Salix arctica* (dom.), *Polygonum viviparum*, *Ranunculus sulfureus*, *Poa arctica*, *Festuca rubra*.

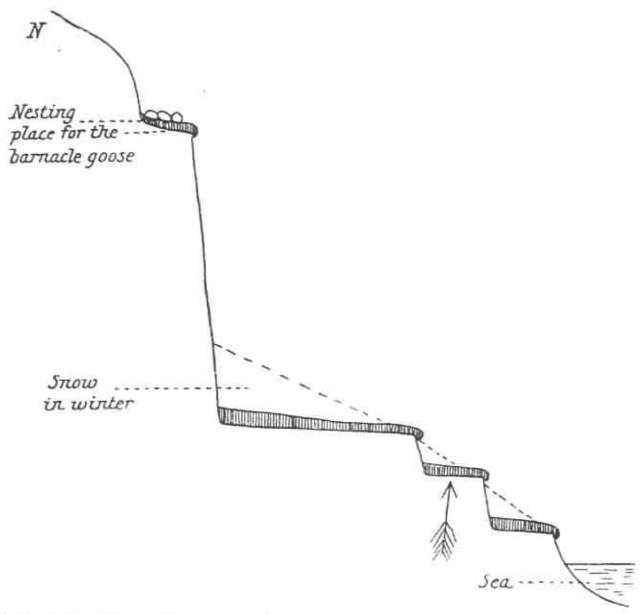

Fig. 1. Eskimonæs, East Greenland; profile view of the locality in which the soil samples were taken (1:400).

Below this there occurred a layer of humus of slight depth, which sent down branches into the underlying layers of clay and sand that have probably been carried to the place by the wind. Lower still there were several layers of humus, presumably indicating old surface layers which have become covered by the clay (loess). These branches are cracks due to desiccation and solifluction, which have been filled up. (Fig. 2).

Greenland, series I.

These samples differed somewhat in size, which amongst other things was due to the fact that it was attended with some difficulty to take samples with a tube of the very stiff clay. The samples were diluted in 14 tubes, with dilution to $^{1}/_{10}$ at each successive stage. Further, agar cultures were established of dilutions No. 1 and No. 2.

It should be noted that the samples were collected on $^{22}/_{8}$ 32 and were not diluted until $^{14}/_{3}$ 33. They had meanwhile become completely desiccated, and it may be surmised that a number of germs had perished in the interval. These samples, therefore, cannot be compared to those collected in Denmark, which were diluted immediately after collection.

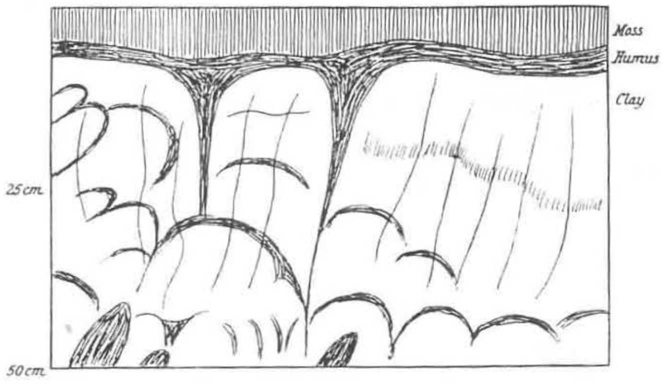

Fig. 2. Eskimonæs, East Greenland; profile view of the soil at the place where the samples were taken (1:10).

As will appear from Table 22, it turned out that there were a great many living germs (333,000) at the surface. The 5—10 cm depth yielded none, the 20 cm depth very few (5), and the 30—40 cm depth rather a large number (5,000—45,000). This series of samples was taken in a place where there was very pure clay without plant remains down to a depth of 5—10 cm, at 20 cm there was a trace of humus in the clay, at 30 cm an admixture of distinct plant remains, while the samples from 40 cm consisted almost exclusively of old plant remains. In this Greenlandish soil animal life is very scarce, notably there is a total absence of earthworms. Hence the factors that contribute to carry algae down into the soil can only be supposed to include 1) water trickling down or 2) the active descent of the algae.

Trickling water will undoubtedly in a soil of this kind (see Fig. 2) follow definite paths on its downward way. It will especially follow the old vertical cracks filled with humus, whereas it will avoid the dense clayey areas. It is therefore natural that very few or no algal germs are found in them. The lower layers of humus which, as previously mentioned, are probably old surface layers that have been covered by clay and sand carried along by the wind, may be expected to harbour living algal germs from the time when they were surface layers, or it may be supposed that the trickling water will continually carry germs down to them. The former alternative is not very probable, however; it is true that the loess deposits here in question are very late formations from a geological point of view, but from a biological point of view they must be regarded as old. The occurrence of the algae at a depth of 40 cm must therefore be supposed to be due to the trickling water. An active descent of the algae is not probable, as shown by my experiments recorded elsewhere.

A characteristic feature of the algal flora in this locality is the great number of Cyanophyceae (8 species). In addition it strikes one as remarkable that these, as well as the Diatoms and green algae occurring here, are in the main the same as may be met with in Danish soils (Table 23).

Table 22. Eskimonæs, Greenland. Series I.

	Size of soil samples c.c.	0	1 a	1 b	2 a	2 b	3 a	3 b	4 a	4 b	5 a	5 b	6 a	6 b	No. of germs pr. c.c. with dilution in		Nature of soil
															liquid	agar	
Surface .	3	11	11	10	8	7	2	4	2	1	2	0	0	0	333.000	75.000	peat
5 cm D.	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	clay
10 —	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	clay
20 —	2	1	0	0	0	0	0	0	0	0	0	0	0	0	5	0	clay
30 —	2	1	1	1	1	1	0	0	0	0	0	0	0	0	500	5.000	clay with humus
40 —	1	5	2	2	2	2	0	1	0	0	0	0	0	0	5.000	45.000	humus

Greenland, series II.

This series, which comprises 4 samples from the surface and one sample from depths of 5, 10, 20, 30, and 40 cm respectively

Table 23. Eskimonæs. Series I.

+ Denotes that the species was only found in dilution No. 0.

	Surface	5 cm Depth	10 cm Depth	20 cm Depth	30 cm Depth	40 cm Depth
Anabaena oscillarioides	1	0	0	0	0	0
— sp.	4	0	0	0	0	0
Cylindrospermum sp........	3	0	0	0	0	0
Nostoc sp.	2	0	0	0	0	2
Oscillatoria sp.	+	0	0	0	0	0
Phormidium autumnale	5	0	0	0	0	0
— foveolarum.......	2	0	0	0	0	0
— tenue............	1	0	0	0	0	0
Hantzschia amphioxys	2	0	0	0	0	0
Navicula Atomus..........	2	0	0	0	0	+
— mutica f. Cohnii ..	+	0	0	0	0	0
— sp.	1	0	0	0	0	0
Pinnularia borealis.........	+	0	0	0	0	0
Stauroneis anceps..........	+	0	0	0	0	0
Bumilleria exilis	5	0	0	0	0	+
Carteria sp.	1	0	0	0	0	+
Chlamydomonas sp.........	1	0	0	0	0	0
Chlorella vulgaris..........	1	0	0	0	0	0
Chlorococcum humicola.....	4	0	0	0	0	2
Dictyococcus minor	1	0	0	0	0	0
Heterococcus viridis........	2	0	0	+	3	4
Hormidium flaccidum	2	0	0	0	0	0
Keratococcus bicaudatus ...	1	0	0	0	0	0
Muriella terrestris..........	1	0	0	0	1	0
Pleurochloris sp............	1	0	0	0	0	0
Stichococcus bacillaris......	4	0	0	0	0	0
Total No. of Species...	26	0	0	1	2	6

was treated in a simpler manner, each sample being put into a Freudenreich flask with 3 c.c. of nutrient medium (according to Bristol's method, half-and-half) and placed in the light. None of these samples consisted of pure clay; in all of them more or less humus was present. The samples were collected on $^{22}/_{8}$ 32; they were inoculated on $^{21}/_{3}$ 33 and were then completely desiccated. All of them yielded algae, the surface samples an average of 12 species each. The total number was 28 species, among which, as in series I, the Cyanophyceae were remarkably numerous (11 species) (Table 24).

Much fewer species developed in the samples from 5—40 cm depth (1—4 in each).

This series of samples furnishes a good supplement to series I. It shows that the occurrence of algae in the lower layers may be dependent on the nature of the soil, and strengthens my conjecture that the reason why algal germs are absent from depths of 5—10 cm is that the water trickling down avoids the areas of dense clay in the soil.

Series II further shows that all the species found in the lower layers also occur in at least one of the surface samples. The only apparent exception here is *Anabaena variabilis*, which was found in an identifiable state in the culture from 30 cm; but it is highly

Table 24. Eskimonæs. Series II.

	Surf.	Surf.	Surf.	Surf.	5 cm	10 cm	20 cm	30 cm	40 cm
Anabæna variabilis.....	..	..	..	..	..	..	..	+	..
— sp.	+	+	+	..	+	..	..	+	..
Cylindrospermum muscicola...............	..	+	..	..	+	..	..	..	..
Nostoc sp............	+	+	+	+	..	..	..	..	..
Oscillatoria anguina....	..	..	+	..	..	..	..	..	..
— janthiphora	+	..	..	..	..	..	..	..	..
— splendida ..	+	..	..	..	..	..	..	..	..
Phormidium autumnale.	+	+	+	+	..	..	..	..	..
— foveolarum	+	..	..	..	..	..	..	..	..
— Valderiæ ..	..	..	..	+	..	..	+	..	..
Symploca muscorum...	..	..	+	..	..	+	..	..	..
Hantzschia amphioxys.	+	+	+	..	..	..	..	..	..
Navicula Atomus......	..	..	..	+	+	..	..	..	..
— mutica f. Cohnii	..	..	+	+	..	..	..	..	..
Stauroneis anceps	+	..	+	..	..	..	..	..	..
Bumilleria exilis.......	+	+	+	+	..	..	..	..	..
Chlamydomonas sp. ...	+	+	..	+	+	..	..	..	+
Chlorella vulgaris......	..	+	..	..	..	..	..	..	..
Chlorococcum humicola	+	..	..	+	..	+	..	..	..
Cosmarium sp.........	..	..	+	+	..	..	..	..	..
Dictyococcus minor....	..	+	..	..	..	..	..	..	..
Heterococcus viridis ...	..	..	..	+	..	+	..	+	..
Hormidium flaccidum..	+	+	..	..	..	..	..	..	..
Keratococcus bicaudatus	..	+	..	..	..	..	..	..	..
Muriella terrestris	..	..	..	+	..	..	..	..	..
Pleurochloris sp.	..	..	..	+	..	..	..	..	..
Stichococcus sp........	..	+	+	..	..	..	..	..	..
Tribonema bombycinum	+	..	..	..	..	..	..	..	..
Total No. of Species ..	13	12	11	12	4	3	1	3	1

probable that some of the species recorded as *Anabaena* sp. from the surface samples were really this species, but in a sterile state, and therefore not identifiable with certainty.

It is shown, then, by these samples from East Greenland

1) that in Greenland, too, there is, in an absolutely virgin locality, on the surface of the soil an algal flora rich in species and individuals, among which the Cyanophyceae are more dominant than in Denmark.
2) that algal germs can also be shown to occur at a depth of 40 cm, though as a rule there are much fewer species and individuals than at the surface.
3) Since animal life, especially earthworms, is virtually absent from the soil of Greenland, these samples show that algae can be carried down into the ground without the help of burrowing animals. This would seem to support the conjecture, set forth by me elsewhere, that it is seeping water which is the main factor in carrying down algal germs into the soil.

V. The Biology of the Soil Algae.

1. The growth of Soil Algae in the dark.

The literature contains numerous statements as to the ability of soil algae to grow in complete darkness as long as certain organic substances are present. We shall briefly go over the information available for the individual groups of algae.

Cyanophyceae.

BOUILHAC in a series of papers (1897, 1898 I and II, 1901) has described his cultivation experiments with *Nostoc punctiforme*. When the nutrient medium contains 1 p.c. of glucose, saccharose, maltose or starch, the alga is able to grow in complete darkness and even produce true chlorophyll (ÉTARD et BOUILHAC 1898). BRUNNTHALER (1909) worked on *Gloeothece rupestris* and cultivated it in light and darkness on a number of different substrata. He arrived at the very strange result that in many of them, amongst others several containing only inorganic nutrient substances, it grew better in darkness than in the light. He speaks nowhere of complete darkness, and it is possible that by darkness he merely means a dim light; otherwise his results seem quite inexplicable, and he himself does not contribute anything towards their explanation. The cultures were very impure, being infected both with bacteria and green algae. It is obvious, too, that the author has been more interested in the morphological than in the physiological aspect.

In contrast herewith PRINGSHEIM (1913, p. 84) arrives at the result that *Oscillatoria tenuis*, *O. brevis*, and *Nostoc* sp. (related to *N. cuticulare* (Bréb.) Born. et Flah.) are unable to grow in the least in darkness, even in the presence of organic compounds which are generally conducive to growth in the light. The investigations of BORESCH (1913) may also be of some interest for the question under consideration. From these it appears that *Phormidium Corium* is able to form chlorophyll independently of the light, as long as nitrates or organic nitrogen compounds are present.

Above we have recorded ESMARCH's (1914) attempts to make Cyanophyceae grow in soil in the dark; we shall point out once more that the result was negative.

HARDER (1917 II) worked again with *Nostoc punctiforme* isolated from the petiole of *Gunnera*. He found that it cannot grow without light in an inorganic substratum; but in substrata containing certain organic substances, especially hexoses, di- and polysaccharides, he ascertained a growth in the dark. HARDER would connect this with the fact that *Nostoc punctiforme* is a species which is specially adapted to live within phanerogams, and therefore equipped with a special power of growing heterotrophically and in the dark.

MAGDEBURG (1933) has found certain Chroococcaceae growing on the walls of subterranean caves in absolute darkness. He explains their occurrence here by the assumption of a symbiotic relationship between them and autotrophic iron bacteria.

Hence, even though it has been asserted of various Cyanophyceae that they are able to subsist heterotrophically and grow in darkness, this can only be regarded as established with certainty for one species, viz. *Nostoc punctiforme*.

Flagellatae.

In the present work I have not occupied myself much with the Flagellates in the soil. The role played by them is not very well known; I shall, however, not omit mentioning some investigations on the physiology of the Euglena species which provide some information that may serve as a guide in estimating the significance of the part which these organisms may be supposed to play in the soil.

ZUMSTEIN (1900) was the first to cultivate an Euglena species viz. *E. gracilis*, in a pure culture; he used it in a series of experiments on the physiology of nutrition which show that it is able to grow autotrophically as well as mixotrophically in the light and heterotrophically in darkness in the presence of suitable organic substances. In the latter case it loses its green colour, the chromatophores being reduced to leucoplasts. If such faded specimens are brought back into the light, they rapidly become green again. These observations have been confirmed and amplified by PRINGSHEIM (1913) and DUSI (1933). The latter has shown, however, that conditions are quite different in the case of other Euglena species. Of the 5 other species with which he worked none was able to grow heterotrophically in the dark even if he offered them all kinds of organic substances. To all five of them light was necessary. It is therefore to be expected that, since the physiological conditions within the genus *Euglena* show such great differences, the differences within the whole class of Flagellates will be still greater.

Thus it is possible that certain green Flagellates may be supposed to be able to grow in the soil. However, according to the information so far available chlorophylligerous forms do not seem to be present in the soil in great numbers.

Diatomeae.

COHN (1854) found a colourless Diatom, *Synedra putrida*, in salt water with putrescent organic substances. It was equipped in all respects as a normal Diatom and moved about briskly, but totally lacked chromatophores. COHN supposes, therefore, that it must live saprophytically and is unable to assimilate carbon dioxide by the aid of the light.

PROWAZEK (1900), BENECKE (1900), and later O. RICHTER (1906, 1911) have recorded similar colourless Diatoms from salt water.

MIQUEL (1892, p. 170) already noticed that under the influence of carbohydrates, glycerine, alcohol, and salts of organic acids Diatoms often become colourless, and he inferred that the presence of these substances enables the Diatoms to live without chlorophyll.

KARSTEN (1901) made a similar observation, especially with *Nitzschia Palea* and *N. amphioxys*; however, the chromatophores never entirely disappeared.

O. RICHTER (1911, p. 39) cultivated *Nitzschia Palea* and *Navicula minuscula* in pure cultures and never observed any reduction of the chromatophores as a result of the presence of organic substances. He thinks, therefore, that this phenomenon in the experiments of MIQUEL and KARSTEN was hardly due to the presence of the organic substances, but must more probably be regarded as a pathologic phenomenon whose incidence was caused by the vigorous development in the organic substance of the bacteria present. MEINHOLD (1911, p. 355), however, made similar observations to those of KARSTEN concerning the reduction of chromatophores, in absolutely pure cultures, when malic acid was present in a comparatively strong concentration. It cannot, therefore, be said to be quite precluded that such colourless forms of Diatoms may occur in soil; but so far nobody has ever seen anything of them.

All investigators (MIQUEL 1892, KARSTEN 1901, RICHTER 1911, p. 39, MEINHOLD 1911, p. 355) agree that certain organic substances can be utilised by brown Diatoms in the light. KARSTEN also thought (1901) that he could show that there was a growth in the dark of brown Diatoms, particularly *Navicula perpusilla*. He employed cultures in hanging drops in which were inoculated a small number of cells, and then counted day by day how many were gradually formed by division. In most cases, amongst other things in the case of *Nitzschia Palea* and *N. amphioxys*, the increase of the number of individuals in the dark was so negligible that it need not denote any real growth (increase of the amount of substance), as when 16 individuals were increased to 21. Only *Navicula perpusilla* showed in some dark cultures a multiplication such as to render it probable that a real growth had taken place, as for instance when 3 individuals increased to 19, or 10 to 32. And even in these cases the experiment was subject to error in that the culture must necessarily have been exposed to the light while the daily examination under the microscope took place, so that these experiments do not seem quite convincing, as has also been pointed out by O. RICHTER (1906, p. 94). The latter, however, in his pure cultures of *Nitzschia Palea* and *Navicula minuscula* (1906) could not show that there had been any appreciable growth in darkness, even in the presence of organic substances. On the other hand, brown as well as colourless Diatoms may be kept a very long time in the dark without any harmful effects. MEINHOLD (1911, p. 356) and TREBOUX (1905, p. 434) also did not succeed in getting brown Diatoms grow in the dark.

As far as we know, then, brown Diatoms are able to assimilate various organic substances in the light, but nevertheless they are not able to live quite saprophytically in darkness.

Chlorophyceae.

KLEBS (1887) was the first to make a green alga grow in the dark. He cultivated *Zygnema* in a liquid medium with sugar and "Eisenweinstein" and gradually succeeded in making it grow in darkness. Later on a multitude of authors have ascertained growth in darkness of green algae, in impure cultures as well as in cultures free from bacteria. A list of this literature has been given by O. RICHTER (1911, p. 26); to this I refer the reader. A number of the species dealt with in this physiological literature are designated by the names of species which may occur as soil algae. In most instances, however, it is not stated that the alga has been isolated from soil, and in many cases it is quite impossible to form any idea as to whether the alga has been correctly identified, since a description of the species of alga dealt with is totally lacking. This has previously been pointed out, for instance by CHODAT (1913, p. 87) and by BRISTOL ROACH (1927 II, p. 509). It is not, however, any very great number of species which has been proved capable of growth in the dark. Apart from KLEBS's experiments with *Zygnema* all the rest are unicellular forms, most of them of the family of Protococcaceae, *Stichococcus*, and some Chlamydomonadaceae, whereas there are Chlorophyceae of very wide distribution about which no information is available in this respect, thus the genera *Hormidium* and *Vaucheria* and the entire group of the Heterokontae. TREBOUX (1905) obtained no growth in darkness of Conferva-, Bumilleria-, and Hormidium species in media containing salts of organic acids.

A general view of the literature on this subject is provided by the list below, arranged according to the names of the algae examined.

Botrydiopsis — HOFFMANN-GROBÉTY 1912.
Chlamydomonadaceae — JACOBSEN 1910, ARTARI 1904—09, 1913—14, TREBOUX 1905.
Chlorella (incl. Chlorothecium) — KRÜGER 1894, BEYERINCK 1898, RADAIS 1900, GRINTZESCO 1903, ARTARI 1904—09, TREBOUX 1905, HOFFMANN-GROBÉTY 1912, KUFFERATH 1913, MENDRECKA 1913, MUENSCHER 1923.

Chlorosphaera — Beyerinck 1898.
Coelastrum — Treboux 1905.
Cystococcus (Chlorococcum) — Beyerinck 1898, Charpentier 1903 I and II, Bristol Roach 1926, 1927 II.
Kirchneriella — Treboux 1905.
Microthamnion — Treboux 1905.
Pleurococcus — Beyerinck 1898, Artari 1899.
Rhaphidium — Treboux 1905, Hoffmann-Grobéty 1912.
Scenedesmus — Beyerinck 1898, Artari 1899, Treboux 1905, Dangeard 1921, Bristol Roach 1926, 1927 II.
Stichococcus — Beyerinck 1898, Artari 1901, 1902, 1904—09, Treboux 1905.
Lichen gonidia — Artari 1899, Korniloff 1913, Jaag 1929.

The main result that emerges from this great number of papers is that most of the green algae investigated are able to assimilate certain organic substances in light as well as in darkness, and thus acquire the ability to grow under the latter conditions. There is, however, a great variation in the organic substances which the various algae are able to utilise. Some species fade in the dark, while others are able to form chlorophyll even in darkness.

The investigations of Bristol Roach (1926, 1927 II, 1928 II), are of special interest since she uses precisely Chlorophyceae isolated from soil for her experiments, and these are especially designed to find out the properties in the algae which might make them particularly adapted to grow in the soil.

The first paper (1926) explains the methods adopted for the pure culture and continued cultivation of the algae. Further, the experimental technique is described. Mrs. Roach uses large flasks (500 c.c.) containing 250 c.c. of liquid medium for her experiments. The neck of the flasks is provided with a side tube with a tap which makes it possible to draw off samples of the culture medium without infecting the culture. Samples of the well-shaken culture were drawn off daily, and in these were found by counting in a counting chamber not only the number of cells per c.c. of liquid, but also the average size of the approximately globular cells. This made it possible to calculate the bulk of the algae contained in the culture flasks with fair accuracy. It then turned out that *Scenedesmus costulatus* Chod. var. *chlorelloides* Bristol Roach grew more rapidly in the light in a nutrient medium containing 1 p.c. of glucose than without this substance; further, it was able to grow in the dark and preserve its green colour in cultures containing 1 p. c. of glucose. The rate of growth in the dark appeared to correspond approximately to the difference between the rate in the light with and without 1 p.c. of glucose, and this would then seem to show that the alga assimilates the sugar at about the same rate in the light and in darkness. Here, however, the third paper comes in (BRISTOL ROACH 1928 II). By a series of experiments on the growth of the same alga in light of varying intensity and partly in a pure mineral salts solution, partly in the same + 1 p.c. of glucose, she arrives at the result that under the most favourable light conditions the alga will grow with about equal rapidity on both substrata, that is to say, it subsists quite autotrophically even in the presence of glucose; whereas with lower intensities of light it will grow more rapidly with glucose than without it; thus with lower intensities of illumination it will begin to assimilate glucose coincidently with the photosynthetic activity. In total darkness it will only be able to assimilate glucose, which results in a rate of growth that is about half that of full illumination. A number of other substances could also be assimilated by the alga (BRISTOL ROACH 1926). These substances, arranged according to their ability to nourish the alga, were: maltose, galactose, "sucrose" (saccharose?), fructose, glycerine, mannite, xylose. It has not been tested, however, whether these substances are also able to nourish the alga in the dark.

The second paper (BRISTOL ROACH 1927 II) deals with experiments with 5 species of soil algae (Protococcaceae) in part only determined for genera. All these algae proved able to grow in the dark in the presence of a suitable organic substance. The organic substances tested were the same as were mentioned above, i. e. all of them nitrogen-free; it turned out, however, that the various substances were of widely different nutritive value for the different soil algae.

These investigations by BRISTOL ROACH are among the most accurate that have ever been undertaken and in so far there is hardly any essential objection to be raised against the correctness of the results. But her attempt to employ these results to prove that algae must be able to grow in soil in the dark takes her on to insecure ground. None of the substances employed in the experiments is of common occurrence in the soil, and here the algae will moreover meet with competitors for the organic food in fungi and bacteria which will always be largely in the majority and be much better able to break down the organic substances than the algae. True, it is possible that certain bacteria may by their activity contribute to liberate precisely such substances, e. g. simple sugars, as the algae could use; but it is probable that at the same time other bacteria will appear and compete against them.

It may be of interest here to recall TREBOUX's investigations (1905). He shows that several species of Chlorophyceae are able to grow in the dark in a solution of inorganic nutrient salts + a salt of an organic acid, e. g. acetic acid. While sugars, as previously mentioned, are hardly found in the soil, organic acids will generally be formed when plant substances decay in the ground. Hence a growth of algae in the soil on the basis of acids and their salts is not inconceivable.

Direct attempts to make algae grow in the soil should be able to clear up this question. Such direct attempts with a green alga were made by MOORE and KARRER (1919, p. 305). They used an alga which they called *Protoderma viride* (according to MOORE and CARTER 1926, p. 104 = *Chlorococcum humicola* (Näg.) Rabh.). This species, they had found, was the commonest in soil down to great depths. The experiment was made in the following way. A glass tube, 2 cm in diameter and 1 m long, was filled with layers of sterile soil alternating with pieces of filter-paper covered with algae from a culture. The top of the tube was closed with a plug of cotton-wool and the whole was buried in the ground so that only the mouth projected a little; this was covered with a flower-pot turned upside down so as to exclude the light, which, however, it was not possible to do entirely. As a result a luxuriant vegetation of algae appeared at the surface of the tube. In the lower part of the tube living green algae were found after 5 months, which had, moreover, spread in the soil; but it cannot be said to be established with certainty whether any growth had taken place here. Another series of experiments, according to a somewhat different principle, gave no certain results either. These experiments have previously been criticised by PUYMALY (1924, p. 57).

In 1932 (III) the present writer published a preliminary paper concerning some experiments undertaken to show whether soil algae are able to grow within the soil in darkness. The experimental technique was as follows. A number of sterile soil samples of 5 c.c. containing 3 c.c. of water, placed in Freudenreich flasks, were inoculated with a known number of germs from a culture of soil algae. Some flasks were exposed to the light, others were kept in darkness, and at certain intervals the flasks from the light as well as from the dark were examined by means of dilution cultures. The algae used for the experiments were *Nitzschia Kützingiana* f. *terrestris* and *Pleurochloris magna.* Of *Nitzschia* 2 series of samples were set up containing respectively 12,500 and 1,250 germs per flask. In both cases it turned out that in the light the Diatoms multiplied very rapidly in the course of 5—7 weeks, so that a vegetation of them was visible macroscopically. I found, too, on counting the germs by means of dilution cultures that the 1,250 germs per flask in series II had multiplied in the course of 5 weeks to 225,000 germs, or upon direct counting in a counting chamber even to 320,000 germs. In a flask that had first been kept in the dark for 22 weeks and then in the light for 13 weeks, 1,780,000 cells were found. On the other hand, it was not possible to show any growth in the flasks from the dark; after 44 weeks in darkness germs to the number of 50—500 were found by dilution (series II).

Of *Pleurochloris* 2 similar series of samples were set up, with 3,130 and 313 cells respectively per flask. In the course of some weeks a vigorous vegetation, visible macroscopically, developed in the flasks placed in the light, whereas nothing appeared in the flasks kept in the dark. After $4^1/_2$ weeks, 1,750,000 cells could be counted in a flask of series II kept in the light. After $4^1/_2$—44 weeks, 50—1000 germs per flask were found in the flasks from the dark. It cannot, therefore, be inferred from these figures that there has been any growth in the dark. In a flask placed first 22 weeks in the dark and then exposed 13 weeks to the light 1,225,000 living cells were found, which makes it clear that all conditions for life and growth, except light, were present in the flasks. The cultures just mentioned were inoculated from algal cultures in which bacteria, too, were present. In the course of the investigations I succeeded in obtaining *Pleurochloris* in a pure culture free from bacteria; so I established a series III of flasks inoculated from this culture. When in 1932 I published the results of my investigations, they had not been completed as far as this series was concerned, so I shall here give a full description of the experiments in question.

Pleurochloris series III.

Flasks inoculated on $^{5}/_{6}$ 31.

In each 5 c.c. of dry soil, 3 c.c. of nutrient medium according to BRISTOL (1920) diluted with an equal amount of water + $^{1}/_{2}$ p.c. of glucose.

Sown: 3,150 cells (from a culture free from bacteria).

3 flasks were left exposed to the light; after some weeks a macroscopically visible algal vegetation appeared in them all.

Number of germs:

Table 25.

Date	Weeks in the light	No. of germs found with dilution in liquid	No. of germs found with dilution in agar	No. of germs found by direct counting
$^{13}/_{7}$ 31	$5^{1}/_{2}$	100,000	315,000	..
$^{10}/_{12}$ -	27	..	..	46,000,000

10 flasks were placed in the dark. No macroscopically visible algal vegetation appeared in any of them. Some of them were subjected to a closer inspection.

Table 26.

Date of dilution	No. of weeks in the dark	No. of germs with dilution in liquid	No. of germs with dilution in agar
$^{13}/_{7}$ 31	$5^{1}/_{2}$	1,000	4,500
$^{17}/_{9}$ -	15	1,000	?
$^{31}/_{12}$ -	30	5,000	?
$^{3}/_{3}$ 32	$38^{1}/_{2}$	1,000	4,000
$^{17}/_{6}$ -	53	10,000	4,000
$^{31}/_{8}$ -	$64^{1}/_{2}$	1,000	2,000
$^{22}/_{2}$ 33	89	500	2,000[1]

[1] A similar flask contained 12.7 p. c. of water.

Pleurochloris might have been expected to multiply in darkness in the soil in the presence of glucose; but the figures in Table 26 do not seem to show this to have been the case. Simultaneously with the examination for Pleurochloris germs of the last flask

($^{22}/_{2}$ 33) the water content of one of the other flasks from the dark was examined and found to be 12.7 p.c., which must be supposed to be still sufficient for a growth of the algae to take place.

A flask which had been kept in the dark for 15 weeks was exposed to the light and after 13 weeks the number of algal cells in it was counted by means of a counting chamber. There were now 5,500,000 cells in the flask.

Thus it is seen that *Pleurochloris magna* requires light for its growth and multiplication, even in the presence of glucose, and therefore behaves differently to several of the green algae investigated by BRISTOL ROACH.

In my preliminary paper (1932 III, p. 21) I mentioned that I had started a series of experiments intending to show how other soil algae behaved when living in sufficiently moist soil and kept partly in the dark, partly in the light.

The experimental technique was as follows. 15 Freudenreich flasks each containing 2 c.c. of tap water were sterilised. 5 c.c. of fresh soil taken in a sterile tube below the chestnut trees in the Botanical Garden were then added to each of them. The soil samples were all taken from a quite small area, uniform as far as possible, and were all collected at the surface. It should be noted that for some nights previous to $^{27}/_{10}$ 31, when the samples were taken, there had been rather sharp frosts, which may have had some influence on the number of germs.

In order to establish how many germs were present in the soil to begin with 2 additional samples of 5 c.c. were taken; these were diluted in 14 tubes in the usual way, and agar cultures were established of 1 c.c. of dilutions No. 1 and No. 2. The results of these dilutions will appear from Table 27.

5 of the 15 Freudenreich flasks were exposed to the light. In all of them there appeared a vigorous algal vegetation of Diatoms, Cyanophyceae, and Chlorophyceae. In one of them mosses also developed and fern gametophytes which even produced a sporophyte and several leaves.

10 flasks were placed in the dark; no macroscopically visible algal vegetation developed in any of them. After 9 weeks, a flask from the dark and one from the light were examined by means of dilution cultures with the result that there was a far greater number of germs (c. 5—10 times as many) in the former than in the cultures started with, while in the latter there occurred

a number which cannot be said to be larger than in those started with. In the succeeding time one of the flasks from the dark was examined at intervals. After a period of 80 weeks the last flask was examined and, as will appear from the figures in Table 27, it then became clear that there could hardly have been any multiplication of the algae in the dark. On the other hand it was found that, after 80 weeks, a very vigorous growth had taken place in one of the flasks from the light, so that the number of algal germs had now risen to 500—1000 times that which was originally present in the soil samples. On counting in a counting chamber this flask was found to contain:

Chlorophyceae		3,000,000
Diatoms	living	2,500,000
	dead frustules	6,000,000
Cyanophyceae		70,500,000

The large number of germs of Cyanophyceae is especially striking. As a rule these algae cannot be separated into their individual cells by shaking, the mucous sheaths surrounding the

Table 27. Garden soil in the light and the dark. Cultures established $^{27}/_{10}$ 31.

See further the text.

Date	Weeks in the light (L) or in the dark (D)	No. of germs found with dilution in liquid	No. of germs found with dilution in agar
$^{27}/_{10}$ 31	0	50,000	95,000
$^{27}/_{10}$ -	0	10,000?	150,000
$^{31}/_{12}$ -	9 D	100,000	90,000
$^{31}/_{12}$ -	9 L	1,000,000	550,000
$^{3}/_{3}$ 32	18 D	100,000	65,000
$^{24}/_{6}$ -	38 D	100,000	52,000
$^{31}/_{8}$ -	47 D	100,000	140,000
$^{22}/_{2}$ 33	68 D	500,000	210,000[1])
$^{19}/_{5}$ -	80 D	50,000	200,000[2])
$^{19}/_{5}$ -	80 L	100,000,000	16,300,000[3])

[1]) Another flask contained 26 p. c. of water.

[2]) Another flask contained 17.8 p. c. of water.

[3]) Found by direct counting: 76,000,000 germs.

trichomes being generally very tough (see Bristol Roach 1927 I, p. 565). In this case, however, the sheaths of *Anabaena* and *Nostoc* were dissolved, so that the trichomes were divided into their individual cells. These two genera, therefore, were very predominant in the dilutions (see Tables 27 and 28).

The flask kept in the dark for 68 weeks showed an unusually large number of germs in the dilution in liquid. This may be mere chance since the method is so rough that merely one germ that has slipped into the flask may make a difference of from 100,000 to 500,000 in the result. In general the highest value will be regarded as the most correct in these dilutions; but since the high figures of this sample are a quite isolated case among all the rest of the samples, they may presumably be disregarded.

Table 28 shows which species have been found in the various samples; and the figures given, the number of tubes in the liquid

Table 28. Garden soil in the light and in darkness. The figures denote the number of tubes in the liquid dilution in which each species occurred.

In the light (L) or in the dark (D)	L	D	D	D	D	D	D	L
Weeks	9	9	18	38	47	68	80	80
Anabæna sp.	0	0	3	1	0	0	0	12
Nostoc sp.	0	0	0	0	0	0	0	10
Hantzschia amphioxys	7	0	0	0	0	0	0	8
Navicula Atomus	6	5	5	0	6	5	4	0
— Borrichii	6	1	0	0	0	0	0	0
Pinnularia obscura	1	0	2	0	0	0	0	0
Bumilleria exilis	7	1	2	0	1	0	0	0
Bumilleriopsis brevis	3	0	0	0	0	0	0	0
Chlamydomonas sp.	1	0	0	0	3	1	3	0
Chlorella sp.	3	0	0	0	0	0	0	0
Chlorococcum humicola	0	2	2	2	2	1	2	0
Dictyosphærium minutum	0	0	0	0	0	1	0	0
Dictyococcus minor	0	0	0	0	0	1	0	3
Fernandinella alpina	3	0	1	0	0	0	0	0
Heterococcus viridis	7	3	0	0	3	6	1	0
Hormidium flaccidum	1	0	0	0	0	0	0	8
Macrochloris dissecta	0	0	1	0	0	0	0	0
Muriella terrestris	2	4	5	8	1	8	3	2
Pleurochloris sp.	2	0	0	0	0	0	0	0
Total No. of species	13	6	8	3	6	7	5	6

dilutions in which each species appeared, give some idea of the proportions in which they occurred in the samples. (See p. 32).

I think, then, that as the result of this series of experiments I can establish that as a general rule algae will not multiply to any appreciable extent within soil in the dark. On the other hand, there is nothing either that would seem to indicate that the number of algal germs in the soil decrease in the dark within the period covered by the experiment.

Summary.

We then arrive at the following results concerning the possibility of algae growing in the soil in darkness.

a. From the literature.

Investigations on the capability of the various groups to grow in cultures in the dark have shown that

1) Cyanophyceae are in most cases unable to grow in complete darkness. For a single species, however, *(Nostoc punctiforme)* growth in darkness has been established with certainty.
2) Flagellates. Of a single species of *Euglena* it has been shown that it can grow in the dark when nourished by nitrogenous organic substances; it then becomes colourless. Other species of Euglena do not possess this ability.
3) Diatoms. Colourless forms of Diatoms are able to grow in the dark; but such forms are not known from soil. Brown normal Diatoms can assimilate carbon in organic compound in the light, but are not able to do so in the dark.
4) Chlorophyceae. As far as a number of true soil inhabitants are concerned it has been proved that they are able to grow in the dark on substrata containing certain organic substances. It has not been proved, however, that these same substances, necessary for such growth, are present in the soil.

b. The present investigations show

1) that a brown soil Diatom, *Nitzschia Kützingiana* f. *terrestris*, and a Heterokonta, *Pleurochloris magna*, are not able to grow in ordinary garden soil in darkness. Nor can *Pleurochloris* grow in the soil without light when glucose is added.

2) that the number of algal germs contained in a soil sample kept in the dark does not increase, whereas, in a corresponding sample kept in the light there develops a luxuriant vegetation of Cyanophyceae, Diatoms, and Chlorophyceae. This would seem to justify the conclusion that the species in question are unable to multiply to any appreciable extent within soil in the dark.
3) That the number of algal germs contained in a soil sample kept in a moist condition in the dark does not decrease, either, within a limited period (89 weeks). This may be due to the great power of resistance of the algal germs; but it may perhaps also be explained in the following way. At the same time as some algal cells perish, others will multiply a little, so that within the period with which we are here concerned, multiplication will about be able to cover the loss by mortality.

2. The Carrying of Algae from the Surface into the lower Layers of the Soil.

The result having thus been arrived at that algae are not able to multiply in the lower layers of the soil, the next question will be how it is that they are, nevertheless, always found in the deeper layers. Before we proceed to answer this question we will report what previous investigators have thought of it.

ESMARCH (1911, p. 78, 1914, p. 257) has arrived at the view that algae (Cyanophyceae) in the soil are carried down passively by various agencies, viz.

1) soil cultivation (ploughing etc.)
2) the activity of animals, especially earthworms.
3) Seepage of surface waters.

He also touches upon the idea, however, that algae must be able to grow in the soil without light (1914, pp. 258, 263). Nevertheless his own experiments (1914, p. 259 ff.) show that filaments of Cyanophyceae buried in the soil are destroyed after some time, with the exception of heterocysts and spores. This he believes is due partly to the absence of light, partly to the »disintegrating« influence of the soil. FRANCÉ (1913, p. 42) thinks that the algae within the soil may receive so much light through the layers of earth that assimilation is possible, hence to him the problem as to how algae are conveyed into the soil does not exist; he imagines them growing there in exactly the same manner as on the surface.

Unlike ESMARCH, MOORE and KARRER (1919, pp. 284, 303) maintain that they have found living algae down to a depth of 1 m, and under such circumstances as exclude that they can be derived from the surface and have been carried down fortuitously to greater or less depths. The localities investigated by them were undisturbed soil of a clayey nature, and holes from worms or other signs that organisms from the surface had been burrowing in the ground were lacking. They infer that the algae must be in a vegetative condition and actually grow in the soil. MOORE and CARTER (1926), BRISTOL ROACH (1920, 1927 III), and GISTL (1932, 1933) take the same view, whereas FEHÉR (1933, p. 178) occupies a special position, assuming, like FRANCÉ, that the soil algae are able to assimilate carbon dioxide photosynthetically even at depths of 10—15 cm.

From the literature, then, we have two theories of the origin of the subterranean algae, viz. that of ESMARCH, according to which these algae are surface algae which have been carried passively down into the soil by various agencies, and the theory of the other authors which is to the effect that algae are actually able to grow and multiply within the soil, either autotrophically or heterotrophically. The former possibility may presumably be regarded as precluded since the light undoubtedly only penetrates a very short distance into the soil. My investigations recorded in the preceding chapter would seem to show that the latter possibility is not correct, either, since it turned out that soil algae are not as a general rule able to grow within the soil in the dark. It will then be natural to try whether ESMARCH's theory will stand the test of a closer scrutiny.

The objection raised by MOORE and KARRER (l. c.) against ESMARCH's theory is that the clay in some of the localities investigated by them was so compact that water trickling down into it would be unable to carry algae with it. The answer to this is that all soil, and especially soil containing much clay or humus, will always in nature be more or less traversed by cracks formed by the alternate contractions and expansions of the soil according to the conditions of moisture. These cracks will alternately open or close in dry or damp weather respectively, and they can be traced far down into the clay. A fine example is provided by the locality in East Greenland mentioned at p. 51. Fig. 2 shows vertical fissures in the loess, partly quite narrow, partly wider ones filled with humus from the surface, in entire agreement with what Ramann (1911, p. 327) has described. Very few algae occurred in the compact blocks of loess; but in the layers containing more or less humus algae were always present, and it is highly probable that these have been carried down from the surface by water sinking down into the soil.

For the sake of completeness I note that frosts, too, may cause a formation of cracks in the soil, especially in a horizontal direction. And, as is well known, frost is a factor that contributes largely to make the soil friable.

We arrive at the result, then, that since the algae cannot grow and multiply within the soil, the theories put forward by Esmarch must most probably be regarded as the correct ones; but in the sequel we shall, in addition, deal with a possibility not previously discussed, viz. whether the algae may possibly pass actively into the soil.

The questions especially to be investigated are, then:

1. Whether the algae pass actively into the soil?
2. Whether water sinking into the soil can carry algae with it?
3. Whether earthworms can carry algae down into the soil?
4. That algae are carried down into cultivated soil when the ground is broken up requires no further discussion.

We shall attempt to answer the three first questions partly through a study of the literature, partly by means of direct experiments.

a. Do Algae pass actively into the Soil?

Data from the Literature.

Most soil algae have motile stages. It is only partly known what external influences determine the direction and intensity of the movements. I shall attempt to report briefly what may be found in the literature about this subject, and the inferences to be drawn concerning the question under consideration.

All the Cyanophyceae of the group Hormogoneae are probably motile in certain phases. In the Oscillatoriaceae the trichomes themselves are motile; in the rest it is generally only the hormogonia that have this ability. Spontaneous motion has also been observed in some Coccogoneae *(Synechococcus)*.

Several authors have observed that the Cyanophyceae are more or less motionless in the dark, thus *Oscillatoria insignis* (FAMINTZIN 1866), *Oscillatoria limosa* (HEGLER 1901, p. 290), *Oscillatoria formosa* (PIEPER 1915), *Oscillatoria* sp. (NIENBURG 1916), *Oscillatoria curviceps* var. (SCHMID 1918, p. 328). This would seem to show that trichomes that have once been carried so far down into the soil that there is no longer sufficient light for them, will not be able to work their way up to the surface again. SCHMID (1921, p. 607), however, found for *Oscillatoria jenensis* that light was not necessary for its movements.

FAMINTZIN (1866) found for *Oscillatoria insignis* that the trichomes turn towards light of a moderate intensity, while they seek to avoid direct sunlight as well as darkness. Similar observations were made by HANSGIRG (1882) for several other species of *Oscillatoria*. He also found that the movements are more rapid in heat than in cold.

Later PIEPER (1915) has investigated phototaxis in *Oscillatoria formosa* and *Phormidium autumnale*. In a light that is not very strong *Oscillatoria formosa* showed positive phototaxis; at a certain optimal intensity of illumination the filaments take up a position at right angles to the direction of the light; but at a more intense illumination they move away from the light. In this PIEPER sees a means by which the *Oscillatoria's* can select for itself the best possible illumination. NIENBURG (1916) has likewise investigated phototaxis in *Oscillatoria* and arrives at very similar results. An abrupt change from light to darkness makes the filament turn and go in the opposite direction. A change from darkness to light, on the other hand, has no influence on the direction of motion.

Very similar observations have been made by HARDER (1918) on hormogonia of *Nostoc punctiforme*, *Anabaena variabilis*, and *Cylindrospermum muscicola* (the latter has no true hormogonia, but the trichomes are motile throughout life). They proved positively phototactic at light intensities ranging from some few to several hundred candles. The light contributes greatly to increase the intensity of movement, notably an increase in the intensity of the light has a strong effect in the case of low degrees of illumination.

In light not unilateral the hormogonia show autonomous movements and amongst other things have the ability to turn round and go in the opposite direction without any demonstrable external cause. Strong unilateral illumination counteracts this and thus makes the hormogonia move in a definite direction. They can be made to turn by external influence, viz. 1) if the apex encounters a mechanical hindrance, or 2) upon a sudden change from light to darkness. As a rare exception GÜNTHER SCHMID (1923, p. 342) only found negative phototaxis in *Oscillatoria Jenensis*, which lives on the ground in deep shade.

These data, then, do not render it very probable that hormogonia should be able to pass down into the soil by their own exertions; they would presumably soon turn back, partly because they encounter mechanical resistance, partly on account of the change from light to darkness.

Chemotaxis in the Oscillatoriaceae has been investigated by FECHNER (1915). He has especially done work on *Oscillatoria formosa*, but also on *Phormidium autumnale*, *Oscillatoria sancta* v. *caldariorum*, and *O. Cortiana*. He has shown that these are susceptible to the influence of certain substances, but they only exhibit negative reactions to chemical stimuli. Free acids, inorganic as well as organic, have a particularly strong repulsive effect. He regards the chemotaxis of the Oscillatoriaceae as a phobotaxis. The stimulus, he thinks, is felt by the apices of the trichomes. (SCHMID (1923), however, has proved this to be incorrect). FECHNER has, moreover, offered a mechanical explanation of the motion of the Oscillatoriaceae which excludes positive chemotaxis. GÜNTHER SCHMID (1923), too, found only negative chemotaxis in *Oscillatoria Jenensis*.

In this connection it may be recalled that ADERHOLD (1888) was not able to show any positive aërotaxis in the Cyanophyceae.

Geotaxis has not been pointed out in any Cyanophycea. STAHL (1884, p. 172), ADERHOLD (1888, p. 322), GÜNTHER SCHMID (1921, p. 608) found that *Oscillatoria's* are quite indifferent towards gravitation.

PRINGSHEIM (1912, p. 297) expresses the conjecture that *Oscillatoria* also possess hydrotaxis, but no investigations on this point are available.

Of less interest for our view of the life conditions of the Cyanophyceae inhabiting the soil are a couple of investigations on the influence of external factors on the rate of motion (GÜNTHER SCHMID 1918, BURKHOLDER 1933).

All in all the investigations here reported do not seem to show that Cyanophyceae to any great extent pass actively into the soil from the surface. They seem to receive their most vigorous incitement to do so from the light, and it is possible that some Cyanophyceae in a strong light may work their way some little distance into the soil; but as a rule they will certainly be stopped partly by the mechanical resistance, partly by the incidence of the positive phototaxis with the low degree of illumination.

From the top mm of the soil they will more probably be induced by the light to come up on to the surface itself. And as a matter of fact this would also seem to be shown by ESMARCH'S method of cultivation which depends precisely on the fact that the Cyanophyceae move up from the soil through a piece of filter-paper by positive phototaxis.

Quite the predominant part of the soil Diatoms are able to move, and it will be only natural to connect this ability with their mode of living (BOYE PETERSEN 1915, p. 298). It is, however, not very well known what it is that directs their movements. A phototaxis was already known by COHN (1867). But the first to explain in more detail the phototactic movements of Diatoms, especially of the *Navicula* species, was STAHL (1880, p. 400). In diffuse daylight they all proved positively phototactic, but with intense illumination, negatively phototactic. STAHL describes how the Diatoms as usual creep backwards and forwards apparently without plan, but after a number of »oscillations« they have nevertheless either approached or receded from the source of light. ENGELMANN (1882, p. 389) has noticed that the movements of *Naviculae* are arrested in the dark and in places poor in oxygen; in the light, however, their power of movement is restored, presumably because they are then able to produce the necessary oxygen themselves. VERWORN (1889, p. 49) has later shown that it is the red rays that are important in this respect, whereas the phototactical stimulus itself is produced by the violet and ultra-violet rays.

VERWORN has moreover observed that not all *Naviculae* respond in the same way to the light, and that the same species can alter its response to light of a definite intensity. When he left *Navicula brevis* exposed to the sunlight for some length of time it showed itself adapted to a higher intensity of light than the specimens that had been kept in semi-obscurity. He believes that they can adapt their response to the light according to the prevailing conditions. On the other hand, *Stauroneis* sp. was normally negatively phototactic. Only in a culture that had been left exposed to the light in the window for 2 weeks did it prove slightly positively phototactic in diffuse daylight and intensely positively phototactic in semi-obscurity. RICHTER (1906, p. 110) too, observed in his cultures distinct positive phototaxis in *Nitzschia Palea* and *Navicula minuscula*. On the other hand, he regards it as doubtful whether negative phototaxis occurs in Diatoms. It is true that he has seen Diatoms move away from the incident light towards the room in agar-cultures in test tubes; but Richter ascribes this to the fact that refraction produced a strong concentration of the light on the side of the tubes turned away from the window, so that the apparently negative phototaxis was more probably a masked positive phototaxis (cf. CHMIELEWSKY 1904). BUDER (1917), however, has asserted that CHMIELEWSKY's criticism of previous authors' experiments on phototaxis is due to a misapprehension. He shows that what determines the direction of the movements of the organisms is not differences in the intensity of the light, but exclusively the direction of the incident rays. It is quite probable, therefore, that the negative phototaxis observed by RICHTER was quite correct.

Hence, as far as our information now goes, the Diatoms roughly speaking exhibit positive phototaxis in a dim light and negative phototaxis in a strong light. There will then be some probability that in strong sunlight the soil Diatoms will try to move down into the soil, but not very far, since there will then with increasing darkness occur positive phototaxis. However, it is far from certain that all species will react in the same manner, there is even a probability that the individual species may to a certain extent alter its response to the light.

ADERHOLD (1888, p. 322) found Diatoms geotactically indifferent, nor could he observe any aërotaxis; but in the dark they ceased to move when there was lack of oxygen, as there may possibly sometimes be in the deeper layers of the soil (cf. ENGELMANN 1882).

Most of the Chlorophyceae inhabiting the soil have likewise motile phases, zoospores and gametes. In the literature Flagellates, especially *Euglena*, are treated together with these, which is quite natural since in respect of the nature of their movements and the external conditions governing them, they have been found to behave like the zoospores. In some few cases only have motile phases of green algae from soil been subjected to investigation. As a rule the forms investigated have been hydrophytic, as these are more easily procured in sufficient quantity for experiments. The observations made on such material cannot of course be directly applied to the soil algae, the less so because it has been found that there is a very great difference in the way in which the zoospores of the different species behave under identical conditions.

Among soil algae subjected to such investigations we may especially mention *Chlamydomonas variabilis* and *Carteria ovata*, originally cultivated from soil by JACOBSEN (1910), and *Botrydium granulatum*.

The relation to the light of the motile cells has been most closely studied. Already for the liberation itself of zoospores from the mother cell light is in many cases necessary, or at any rate it contributes to further this process (STRASBURGER 1878, p. 14). The present writer (BOYE PETERSEN 1932 III, p. 405) has himself observed in a typical soil alga, viz. *Pleurochloris magna*, that the zoospores are formed in the cells after 48 hours in the dark; but they are not liberated until exposed to the light. Here they only swarm for a short time. How long the motility of zoospores lasts is again largely dependent on the light. Some organisms are positively, others negatively photokinetic (BOLTE 1920). Photokinesis was first observed by ENGELMANN (1882) in purple bacteria which move chiefly in light only, but come to rest in the dark. STRASBURGER (1878, p. 52) has found negative photokinesis in *Ulothrix zonata*, *Botrydium granulatum*, and *Haematococcus lacustris*, BOLTE (1920) in various Flagellates, *Chlamydomonas* species, and *Ulothrix subtilissima* (which is possibly a soil alga). Negatively photokinetic swarmers will continue to swarm in the dark; in some cases (*Haematococcus*) STRASBURGER saw them swarm until they died; in the light, however, such swarming cells will rapidly come to rest and attach themselves. In this circumstance BOLTE (1920, p. 321) sees a means for furthering the settling of the zoospores in a good light, in a place favourable to their continued development. As far as the soil algae are concerned, it will evidently signify that the zoospores of species with a negative photokinesis will only come to rest on the surface of the soil, not in the deeper layers.

Other green organisms, on the other hand, show a positive photokinesis; i. e. they are motile in the light but come to rest in the dark. This applies to *Chlamydomonas variabilis* and *Carteria ovata* amongst others, as also to several Volvocaceae (BOLTE 1920, p. 300). How the positive photokinesis should be explained from an ecological point of view is, according to BOLTE (l. c. p. 322), not at all clear. The positive photokinesis of *Chlamydomonas variabilis* and *Carteria ovata* may, however, be conceived to be connected with the fact that these species are generally found within the soil, since they come to rest precisely in the dark.

A number of organisms, especially colourless ones, are photokinetically indifferent. Photokinetically sensitive organisms are as a rule also phototactic.

On phototaxis there exists an extensive literature. We shall call attention to some main points. It is probable that all light-sensitive (photometric, STRASBURGER 1878), organisms can exhibit positive as well as negative phototaxis, and as a rule they are positively phototactic in a dim light, negatively so in a strong light. The degree of illumination at which the change takes place is different for the different organisms, and likewise differs for the same organism under different external conditions. Thus FAMINTZIN (1866) found that *Chlamydomonas pulvisculus* and *Euglena viridis* originating from a pool containing rather highly saline water were negatively phototactic in bright sunlight, positively phototactic in diffuse daylight. If, however, the water in which they were generally to be found was replaced by water from the Neva, their response was altered so that they became fairly indifferent, though mostly inclined to be negatively phototactic even with rather a low degree of illumination. FAMINTZIN further found that not all individuals showed identical behaviour under identical circumstances. STRASBURGER (1878, p. 17) made similar observations with zoospores of *Ulothrix zonata* and *Haematococcus lacustris*, whereas the gametes of *Botrydium* always proved positively phototactic. He found, moreover, that in a dish with swarming spores of *Ulothrix* a certain percentage were negatively phototactic, even though the majority reacted positively. His explanation of this is that the young zoospores chiefly respond positively, whereas the older zoospores respond negatively to diffuse daylight, and, indeed, he observed that the negatively phototactic swarmers are most inclined to come to rest and attach themselves. STRASBURGER (l. c. p. 41) finds zoospores of *Vaucheria* totally indifferent to the light. It is not known whether this also applies to the species that especially inhabit the soil.

Various external factors have proved capable of influencing the phototactic response of the zoospores. Thus BUDER (1917, p. 111) has shown that *Chlamydomonas variabilis* and *Carteria ovata* may give both a positive and a negative response to light of the same intensity. In the original cultures, where in addition to these organisms numerous bacteria were present, they both showed positive phototaxis; whereas, when he gave them fresh water to move in, they showed marked negative phototaxis. He thinks that it was due to the different oxygen content of the liquids, these algae being thus positively phototactic in liquids poor in oxygen, but negatively phototactic in liquids rich in oxygen. (STRASBURGER (1878, p. 63) already observed something similar).

MAINX (1929) has shown that the phototactic response, especially of *Synura*, can be influenced in a definite direction by changes in pH, temperature, (cf. also STRASBURGER 1878, p. 55), surface tension, anaesthetics, and perhaps also by photokatalysts. It must be taken for granted that the response of the motile stages of the soil algae may likewise be influenced by external factors; but it is of course quite uncertain whether they are affected in the same way as *Synura*. STRASBURGER (1878, p. 34) arrives at the result that it is the direction of the rays and not the intensity of light which determines the direction of the phototactic movements, and BUDER (1917, p. 189) adopts the same view. Unlike him OLTMANNS (1917) and PRINGSHEIM (1912, p. 188) think that the photometric organisms are able to seek out the places which have the best light intensity for them.

In addition to the phototactic movements mentioned above, called by PFEFFER (1904, p. 755) topotactic, there exist another kind of movements, called phototactic by the same author, and which are the reaction of the organisms to a sudden change in the intensity of the light. In the phobotactic movement the organism suddenly starts back and turns round no matter in what direction it was moving before. At the present stage of our knowledge it is difficult to form any idea of what part phototaxis can play in the movements of the zoospores of the soil algae.

Only a very few green algae (including Flagellates) have been investigated with respect to their relation to gravitation. SCHWARZ (1884), however, has pointed out a distinct negative geotaxis for *Euglena viridis* and *Chlamydomonas pulvisculus;* both are able to work their way up through a fairly thick layer of sand ($1^1/_2$—3 cm) in the dark. ADERHOLD (1888) could confirm this for *Euglena* and found that *Haematococcus lacustris* and swarmers of *Ulothrix tenuis* behaved in the same way as *Euglena*. With respect to the swarmers the negatively geotactic reaction was only distinct while they were young, and since they quickly came to rest, it proved difficult to observe them more closely. Later on the same thing was observed by MASSART (1891) for *Chlamydomonas* and by JENSEN (1893) for *Euglena viridis*. MAINX (1929, p. 162) also found marked negative geotaxis in *Synura*, *Volvox*, and *Eudorina* under normal conditions. The response is intensified by the addition of acid or alkali. All these investigators allege that the geotactic reaction is only observed distinctly when other stimuli, such as phototactic and aërotactic, are excluded. Hence, strictly speaking we do not know how the swarming cells of the soil algae behave, but there is some probability that they will all be found to exhibit a more or less marked negative geotaxis, and accordingly in the deeper layers of the soil will have a tendency to work their way up towards the surface.

No rheotaxis has been shown to be present in algae; ADERHOLD (1888, p. 313) mentions that he found no indication of rheotactic sensitivity in *Euglena viridis*.

We owe to PFEFFER (1884, 1888) the fundamental investigations on chemotaxis. He, however, did most of his work on spermatozoids, bacteria, and colourless Flagellates. In green Flagellates (1888, p. 597) he found no chemotactic irritability (amongst other things in *Euglena viridis*, *E. oxyuris*). On the other hand, he observed such irritability in *Chlamydomonas pulvisculus* (l. c. p. 613), which was attracted by various substances, as potassium chloride, rubidium chloride, dextrine, asparagine, peptone, meat extract. Strong solutions of these substances, however, had a highly repulsive effect.

FRANK (1904, p. 174) likewise found in a *Chlamydomonas* (*C. tingens*) pronounced positive chemotaxis to nitric acid and nitrates, phosphoric acid and phosphates, but not to alkalies; ammonia proved highly repulsive. Organic substances such as cane sugar, glucose, glycerine, asparagine were inactive, whereas meat extract proved highly attractive. Carbonic acid was likewise attractive. Further, FRANK has worked with *Euglena gracilis* in the green as well as the colourless form. He finds that both of them showed a chemotactic reaction to the same substances. Unlike the *Euglenae* mentioned by PFEFFER, *E. gracilis*, according to FRANK, exhibited strong chemotaxis.

It would have been of special interest to know whether the soil algae show any chemotactic reaction to oxygen (aërotaxis). On this subject, however, very little information is available. ADERHOLD (1888) observed positive aërotaxis in *Euglena viridis*; JENSEN corroborates this and has seen something similar for *Chlamydomonas pulvisculus* (1893, pp. 449, 453). On the other hand, FRANK's attempt (1904, pp. 179, 185) to point out aërotaxis in *Chlamydomonas tingens* and *Euglena gracilis* has given no certain results.

Thus our knowledge of the chemotaxis of the soil algae is very slight; yet we must suppose that their movements may be influenced by attraction or repulsion of certain substances, and it cannot be said to be inconceivable that chemotactic effects may in certain cases make swarming cells attempt to penetrate into the soil, but possibly also in other cases make them move upwards towards the surface.

In conclusion we will try to sum up what inferences may be drawn from the literature on the locomotion of the algae as to the possibility of their working their way actively into the soil. We cannot arrive at any certain result since investigations on some of the tactic reactions which might, precisely, be thought to contribute most to a descent, are lacking. I am here thinking especially of hydrotaxis, and the available investigations on geotaxis are likewise deficient. From the information available it would seem that the algae are negatively geotactic, and would, therefore, work their way upward. Several observations go to show that the phototactic reactions are much more vigorous than the responses of the other stimuli and so will be capable of greatly minimising the effect of the latter. (JENSEN 1893, p. 449, PFEFFER 1888, p. 596, PRINGSHEIM 1912, pp. 92, 286). In nature, therefore, it will presumably be the phototactic reactions which will determine the direction in which the surface algae move: with regard to the algae within the soil other stimuli will be decisive. All the algal groups under consideration show positive phototaxis with a low degree of illumination, negative phototaxis in a strong light. At what point the change sets in is, however, very different for the different species, and likewise for the same species under different external conditions.

There is, then, no reason to suppose that they should descend very far into the lower layers of the soil as a result of phototaxis. Even if other tactic reactions, especially hydrotactic and chemotactic (trophotactic), may be supposed to have the effect of drawing them downward, they will hardly be able to preponderate over the effect of the phototactic stimulus.

For algae in the deep layers of the soil negative geotaxis and positive aërotaxis will presumably be the chief factors that will induce them to wander upwards, while hydrotaxis, if it exists, must more probably be thought to act in the opposite direction.

It is a reasonable assumption that as a result of the tactic movements of the soil algae they might accumulate at a certain slight depth below the surface where there is a very dim light and a suitable amount of moisture and oxygen. Such a phenomenon has actually been observed, especially when the soil consists of sand which allows the light to penetrate some way under the surface. I shall here remind the reader of the sand algae at the seashore mentioned by WARMING (1904, p. 23, 1906, p. 134), which are never found at the surface but always some slight distance below it (1—2 mm). DÉHÉRAIN et DEMOUSSY (1900, p. 466) have also described how the algae in the sand of their cultures were chiefly found at a depth of one cm; but the algae wandered up and down according to the degree of illumination, as long as the sand was moist enough not to prevent their movements. When the sun shone for several days they all wandered downwards, so that the surface of the sand became quite white; but when the sun was hidden behind clouds for several days, the algae again appeared on the surface.

Soils containing much humus will probably be so impenetrable to the light that here the algae as a result of the conditions of illumination will mostly tend to inhabit the surface itself. This would also seem to be confirmed by observations in nature, where in the great majority of cases I have found the algae there. As is well known, a number of algae, especially green algae, are unable to move in their usual shape, and are accordingly bound to the place where they have once settled.

The great numbers of algae observed by BRISTOL ROACH (1927 II, p. 572) at a depth of 4—6 in. have probably not accumulated there in the way just mentioned (see later on). FRANCÉ (1913, p. 43) has asserted that Diatoms chiefly occur some distance below the surface, but I have not found this corroborated by my investigations.

Hence no observations have rendered it probable that algae should work their way actively down into the soil as a result of external influences.

In order to put this question ,to the test I have, however, made various experiments which will be described below.

The writer's own experiments.

The glass tubes used for the experiments were about 5 cm in diameter and about 20 cm long. They were provided at the bottom with a well-paraffined cork plug, introduced while warm. The sides of the glass were then covered both inside and outside with several layers of aluminium foil as far down as possible (to about 5 cm from the plug) and in such a way that the upper edge of the glass was also covered. The object of this was to prevent light from penetrating through the glass walls. Then the whole of the tube was wrapped up in black paper from the bottom to about 5 cm from the upper edge, and the paper fastened with string in two places. The tubes were then filled with well moistened soil up to a couple of centimetres from the top which was covered with a glass dish turned upside down. The soil was now quite excluded from the light with the exception of the free surface.

The tubes were sterilised for half an hour in an autoclave at 120°. In the middle of the free surface was dripped a drop of water containing a certain number of algal germs from a culture, or 5 c. c. of fresh soil from the surface containing an unknown number of germs were added.

The glass tubes were now placed in the light and after a certain time a growth of algae was seen in all of them. In those cases in which the tubes had been inoculated with germs from a culture it was ascertained by microscopic examination that it was really the algal species under consideration which had developed on the surface of the soil.

After being left to stand for a shorter or longer time, the plug was carefully removed from the tubes, and with a sterile glass and in a sterile way a soil sample of 5 c.c. was taken from the bottom of the tubes, and diluted in the usual way in 14 tubes with Bristol's nutrient medium, while 1 c.c. of dilution No. 1 was diluted in agar. At the same time the percentage of water still contained at this time in the soil at the bottom of the tube was determined.

When moist soil is autoclaved in a glass tube as described above it has a tendency to contract a little after cooling so that a narrow space will appear between the soil and the glass walls. In order to exclude the possibility of the algae making their way down through this intervening space the soil was pressed firmly against the glass walls with a sterile spatula before the inoculation in tubes Nos. 7—12. This precaution, however, proved unnecessary, since the algae did not descend to the bottom in tubes Nos. 1—6 either, though they had not been treated in the abovementioned way.

Table 29. Experiments on the capacity of soil algae to grow actively down into the soil.

Nr.	Date of inoculation	Species of alga	No. of germs	Date of dilution	Percent. of water	Germs found at the bottom	Number of weeks left to stand
1	$^{7}/_{10}$ 31	Pleurochloris	264.000	$^{14}/_{3}$ 32	19,7	0	22
2	$^{7}/_{10}$ -	Nitzschia	8.000	$^{20}/_{3}$ -	19,2	0	23
3	$^{7}/_{10}$ -	Hormidium	some filaments	$^{8}/_{3}$ -	..	0	21
4	$^{7}/_{10}$ -	Dictyococcus	104.000	$^{20}/_{3}$ -	19,1	0	23
5	$^{7}/_{10}$ -	Pleurochloris	264.000	$^{14}/_{3}$ -	19,7	0	22
6	$^{7}/_{10}$ -	Nitzschia	8.000	$^{10}/_{3}$ -	19,7	0	21
7	$^{22}/_{10}$ -	Pleurochloris	272.000	$^{17}/_{3}$ -	18,1	0	16
8	$^{22}/_{10}$ -	Pleurochloris	272.000	$^{4}/_{6}$ -	15,8	0	28
9	$^{22}/_{10}$ -	Pleurochloris	272.000	$^{4}/_{6}$ -	15,1	0	28
10	$^{22}/_{10}$ -	Pleurochloris	272.000	$^{17}/_{3}$ -	16,8	0	16
11	$^{22}/_{10}$ -	5 ccm garden soil	?	$^{8}/_{3}$ -	16,7	0	$16^{1}/_{2}$
12	$^{22}/_{10}$ -	5 ccm garden soil	?	$^{24}/_{6}$ -	14,0	0	$30^{1}/_{2}$

The result of these experiments was that in no case were algae found at the bottom of the tube after 16—$30^{1}/_{2}$ weeks. The percentage of water in the soil varied from 14.0 to 19.7 p.c. according to the time the tubes had been left to stand, thus an amount of water fully sufficing for a growth and descent of the algae to have taken place.

Of the algae chosen for the experiments it might be surmised that they would be able to make their way down into the soil actively, if it were in their nature to do so. Most of the experiments were made with the Heterokonta *Pleurochloris magna*, which is able to form zoospores. Of Chlorophyceae I used *Hormidium* sp. (1 experiment) which likewise, though rarely, will form zoospores, and *Dictyococcus irregularis* (1 experiment), which exhibits a brisk formation of zoospores. Finally the Diatom *Nitzschia Kützingiana* f. *terrestris* (2 experiments) has owing to its power of motion the possibility of working its way into the soil of its own accord.

In the garden soil (2 experiments) algae of all groups will be found, thus Heterokontae, Chlorophyceae, Diatomeae, as well as Cyanophyceae. These latter have motile phases in their hormogonia, which might have caused a descent of these algae on this occasion.

The experiments undertaken to check those mentioned in the sequel on the effect of seeping water on the algae further support these experiments (pp. 94, 96, 99, 101). For in these, too, no algae appeared at the bottom of the tubes. This is of interest because the soil in these control experiments contained more water, viz. 21. 9—25.3 p. c., which is about as much as the earth is capable of absorbing. Further these tubes were left in the dark, which may be conceived to be of importance, especially with respect to the formations of zoospores.

The experiments would then seem to indicate that the soil algae do not descend actively into the soil, but are conveyed passively into it by other agencies.

b. The Bearing of Water Seepage on the Descent of Algae into the Soil.

Among the agents that might contribute to carry algae downward from the surface into the ground, seeping water might at the outset be imagined to be one of the most important. But the filtering power of the earth might be supposed to make itself so much felt that the algae were only very slowly carried down into the ground. The heavier a shower is, the greater must its power in this respect be supposed to be, since the drops, striking the surface vigorously, may loosen germs from layers of algae otherwise rather compact and coherent, and split up clumps of algae in their individual cells. And at the same time, the rate at which the water soaks through the soil will presumably be as great as possible and it will thus be able to carry algal cells down with it. On the other hand, it is probable that small showers and softly-falling steady rains can do this to a less degree. It must be supposed, however, that the various soil algae, according to their whole structure, are very differently suited for being thus washed down. Some algae will in their usual shape easily be carried along with the rain water, thus many Chlorophyceae, Heterokontae as well as Isokontae, viz. all unicellular species without mucilage envelopes. As examples I may mention *Chlorella*, *Pleurochloris*, *Heterococcus*, and *Stichococcus;* and these genera are in fact abundantly represented in the subterranean algal flora. Many of these green algae may also produce zoospores, of which more below. The Cyanophyceae, whether they belong to the Coccogoneae or the Hormogoneae, are as a rule provided with more or less tough mucous sheaths which keep the cells together in larger aggregates, fasten them to particles of soil, and thus prevent the water from carrying them along. These algae, however, are able to produce various kinds of spores, some can produce free individual cells, or small cell complexes which can presumably easily be washed down. GEITLER (1930, p. 25) thus mentions endospores, exospores, hormogonia, planococci, gonidia, resting cells, and hormocysts. Many of these cells even possess motility. It is not known what induces the plants to form these organs of reproduction. Sometimes the mucous envelopes around the cells may be dissolved, by which process the latter are liberated; I have mentioned such a phenomenon at p. 67. In a soil culture exposed to the light the *Nostoc* and *Anabaena* filaments were after 80 weeks found to be dissolved into their individual cells, which in dilutions on agar proved perfectly vigorous. It is not improbable that a similar dissolution of the filaments may occasionally occur in nature. It has not been investigated what is the cause of such a dissolution of the mucous sheaths (see, however, p. 89 (VISCHER 1926)).

The Diatoms, it is true, may form long chains by division; but these are nearly always very loosely coherent only, and will readily be separated by a shower into individual cells which will then easily be carried down with the water. It must be supposed, however, that Diatoms that creep on the particles of the ground will also adhere somewhat to them, which will counteract the washing down of them.

Not a few green algae form large cell aggregates held together by a mucous envelope. We may here recall especially *Mesotaenium*, *Gloeocystis*, *Coccomyxa*, *Chlamydomonas*. If such algae have not the power to liberate their cells under certain circumstances at the dissolution of the mucilage or by the formation of zoospores, they will only with great difficulty be washed down into the soil by the water. Among the terrestrial algae mentioned there are several, e. g. *Mesotaenium*, which have not so far been found in the lower layers of the soil; but this may of course be due to other circumstances, they may amongst other things have been unable to grow on the substrata employed in the cultures. Further, many terrestrial Chlorophyceae are filamentous, and they cannot then be quite simply washed down into the soil. Some of them, e. g. *Vaucheria*, have not hitherto been found in the lower layers, and this may amongst other things be due to the fact that neither their filaments nor their zoospores or oospores are likely to be carried along with the water, the filaments on account of their cohesion, the spores on account of their size. But several filamentous Chlorophyceae, especially Ulothricaceae and Tribonemaceae have been found below the surface. These filamentous algae have then as a rule two ways in which they can form stages able to be carried down by water seepage, viz. 1) the splitting up of the filaments into single cells, and 2) the formation of zoospores.

A. BRAUN (1851, p. 140) already saw that *Hormidium* on land could under certain circumstances divide its filaments into single cells and multiply in that way. Later on this has turned out to be a very common phenomenon among filamentous algae. I shall here merely give some particulars of the available information concerning the fragmentation of the filaments of soil algae, the explanation of the mechanics, and the causes of its incidence.

GAY (1891, pp. 61—63) has mentioned the fragmentation of the filaments in *Ulothrix dissecta* and *U. flaccida* (*Hormidium d.* and *H. f.*), which behave differently, the filaments of *U. dissecta* becoming dissociated in humid air, whereas they remain entire in *U. flaccida*. Submerged, the cells of *U. dissecta* will be transformed into hypnocysts, while the filaments of *U. flaccida* fall into smaller pieces. GAY attempts no explanation of how the dissociation is brought about.

KLEBS (1896) studied the fragmentation in *Hormidium nitens* (p. 331), *H. flaccidum* (p. 341), *Conferva* (p. 374), *Bumilleria exilis* (p. 390), and *B. sicula* (p. 379). He found that the conditions for the incidence of dissociation are in the main the same for all these species, but that it occurred more or less readily in them. The circumstances that may bring about fragmentation are:

1. Slow desiccation.
2. Lack of nutrient salts.
3. The process only seems to occur in the light.

As to the cause of the splitting KLEBS supposes that the turgescence of the cells increases under certain external conditions, viz. when the growth is arrested but not the production of matter. The cells are then rounded at the apices and separated from each other.

BENECKE (1898, p. 455) distinguishes between a rapid and a slow incidence of fragmentation. He has been able to produce the former experimentally by subjecting the cells to the most different influences; the result of these actions was in all cases a sudden increase in the turgidity of the cells, which then resulted in the dissociation of the filaments.

He thinks, on the other hand, that slowly occurring fragmentation may be due to other circumstances, more especially gelatinising of the outermost layers of the wall and a special growth of the septa. In nature one would be most likely to expect this kind of fragmentation; but the former kind may perhaps also occur. However, there does not seem to be any sharp line of demarcation between these two kinds of dissociation. PIERCY (1917, p. 518) has studied the splitting of the filaments in nature in a form of *Hormidium flaccidum*. Her observations do not quite agree with those of KLEBS. She has seen no dissociation occur as a result of desiccation alone. Only when the filaments are moistened again, does dissociation occur in the course of a few days. She thinks, therefore, that it can hardly be due to changes caused by turgescence, and as a matter of fact she shows that before the dissociation occurs, a gelatinous substance is formed in the septa, which facilitates it. Since this substance is not formed in all the septa, splitting does not occur between all the cells. VISCHER (1926,

p. 188) in accordance herewith points out (without, however, quoting PIERCY's work) that the mechanical cause of the dissociation of the filaments may be

1. that the cells are packed with the products of assimilation, become rounded, and thus break down the resistance of the walls;
2. that the septa between the cells for some reason or other become mucilaginous and lose their power of resistance without any change of the cell content.

He finds this latter effect in *Pseudendoclonium basiliense*, *Chlamydomonas pulvinata*, *Scenedesmus basiliense*, and *Stichococcus bacillaris*. He shows that the substances which are able to make colloids imbibe water, favour the separation of the cells, whereas substances preventing the swelling of the colloids also prevent the dissociation. As substances of the former kind are mentioned maltose and glucose, of the latter saccharose and lactose. Special conditions seem to be present in the terrestrial form of *Zygogonium ericetorum*, in which WEST and STARKEY (1915) observed 1) that filaments placed in water are split up into short pieces which then grow out into long threads consisting of thin walled cells; 2) that if the alga is subjected to slow desiccation, the filaments will fall to pieces in thick-walled "cysts" (separate short pieces consisting of few thick-walled cells); 3) that such "cysts" are also formed at low temperatures.

It must then be taken for granted that the causes of the dissociation of the filaments may differ. In nature, and in the case of the soil algae, however, the frequent changes in the conditions of moisture and in the concentration of the nutrient solution will no doubt favour this process, and it must be assumed that the filamentous algae which have the power of dissociating their filaments do this under normal circumstances too. This renders it possible for these species to be washed down into the soil with the water.

Most of the green algae that inhabit the soil, both Isokontae and Heterokontae, are able to form motile cells, zoospores or gametes (whether neutral or sexual is of no great importance in this connection, so in the sequel they are mentioned en bloc). Such cells will evidently be well suited to be washed down with water into the lower layers of the soil; we do not know whether they, with their motility, will resist such a passive conveyance;

but it is probable that the water of heavy showers will be able to overcome such resistance.

Numerous statements concerning the conditions for the formation of zoospores in the algae are found scattered throughout the literature, and these statements are often, apparently at any rate, contradictory. This, however, is probably due to the fact that 1) the individual species, even nearly allied ones, differ in this respect (FREUND 1908, p. 75), 2) that the same species under different external conditions does not always show the same reaction to a new factor, but its response to such a factor will depend on the other factors present; 3) it is possible that within what we now regard as species there are really minor species which are totally different precisely in respect of zoospore formation (see below on *Hormidium* forms). We must then take it for granted (cf. FREUND 1908, p. 43) that special external conditions for the formation of zoospores have only the desired effect when certain general external conditions are present, just as the momentary internal condition of the algae (age, state of nutrition) may decide whether zoospore formation will occur under the given external conditions. This circumstance renders it difficult to view the available observations under the same aspect.

What is of interest to us here is then merely what is known about the zoospore formation of the soil algae in particular, whereas the observations of this process in hydrophytic algae cannot be applied to these.

The most important and thoroughgoing investigations on zoospore formation are due to KLEBS (1896), who has included in his studies some of the soil algae, viz. *Vaucheria repens*, *Protosiphon botryoides*, *Botrydium granulatum*, *Hormidium nitens*, and *H. flaccidum*, *Bumilleria sicula* and *B. exilis*. When these algae grew on soil ("Lehm") they all formed zoospores if water was poured over them; if they grew in KNOP's nutrient solution and were then transferred to clean water, they behaved in the same way. In some cases transference to the dark alone was able to start zoospore formation, in other cases not. Light, on the other hand, nearly always proved an inhibiting factor. It is another thing that light is in many instances necessary for the liberation of the zoospores, or at any rate stimulates it (STRASBURGER 1878, p. 15, VISCHER 1933, p. 15, GERNECK 1907, p. 283). GERNECK (1907, p. 283) found, too, that various soil algae were very apt to form zoospores when they were transferred from a solid substratum to a liquid medium, or better water, just as he also found that transference from a liquid nutrient medium to pure water and darkness furthered zoospore formation. Of soil algae he cultivated *Planophila laetevirens*, *Chlorotetras asymmetrica*, *Ophiocytium breve* (= *Bumilleriopsis brevis*), *Gloeocystis vesiculosa*, *Conferva bombycina*, *Stichococcus flaccidus* (= *Hormidium f.*), *Stichococcus subtilis* (= *Hormidium nitens*), *Monocilia viridis* (*Heterococcus v.*), which all of them more or less readily formed zoospores in the ways mentioned above.

It would then seem to be a very common phenomenon, especially among the soil algae, that they form zoospores when they are transferred to water, and in all green algae the formation of zoospores appears to be common when they are transferred from a nutrient liquid to pure water. A shower must therefore be supposed to have the effect on the soil Chlorophyceae of starting a zoospore formation, both because it moistens the algae and at the same time suddenly dilutes the nutrient solution.

There seems to be a certain reciprocal relation between the power of filamentous algae to become dissociated into individual cells and the formation of zoospores, so that when one of these powers is strongly developed, the other is present in but slight degree, and the reverse. This is already plainly seen in two of the *Hormidium* forms studied by Klebs (1896), forms which he calls *H. flaccidum* and *H. nitens*, the former of which readily forms zoospores, but is only with difficulty made to dissociate its filaments, whereas the latter carries out this process on the slightest provocation, but on the other hand very rarely forms zoospores. Piercy (1917, p. 523) has studied a third form of *Hormidium*, whose filaments were most readily dissociated in nature; but she was not able to induce it to form zoospores by any means. *Stichococcus bacillaris* may also serve as an example of such a form which has a pronounced power of dissociation, but cannot form zoospores at all.

As will be seen, it may in part be the same external influences which make various species either dissociate their filaments or form zoospores, and it must be supposed that showers will always act in one of these ways on the soil algae. There is reason to suppose, therefore, that green algae, too, of the filamentous form or in mucous aggregates, will often during showers pass into such a form that they can be carried down into the soil with the water.

It can further be conceived that it may be of importance for the conveyance of algae through the soil whether or not it is riddled with earthworm burrows, and in the former case it may be supposed that the penetrability of the soil to the algae will be greatly increased.

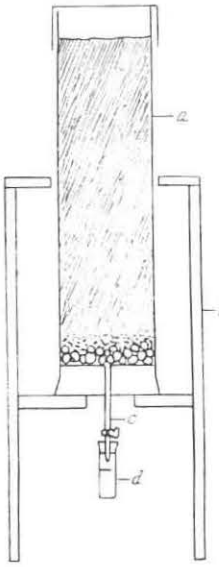

Fig. 3. Apparatus for seepage experiments.

I have made a series of experiments which, briefly, were designed to test whether it would be possible to convey algal germs through the soil by dropping water with the same intensity as that of a natural shower on to the surface of a column of earth.

For this I employed (Fig. 3) glass tubes (*a*) about 5 cm in diameter and about 20 cm long. They were provided with a tightly fitting cork plug furnished with a glass tube (*c*) with a cock; this was fitted with a cork plug on which a test tube (*d*) could later be fitted. The end of the glass tube *c* and the cock were prior to sterilisation wrapped up in paper which was removed immediately before the experiments were started, when a sterile test tube (*d*) with a scale division of 5 c.c. was placed on the tube. The bottom of the glass *a* was covered with a layer of pebbles and over this a layer of sand, in all about 3 cm. The remaining part of the tube was filled with well-moistened garden soil up to about 1 cm from the edge. The tube was covered with a glass dish turned upside down. Before the sterilisation, water was dripped in from above until the water content of the soil was as near as possible to the point of saturation. This caused the earth to sink somewhat, and a fairly firm coherent layer was formed on the surface. After sterilisation ($^1/_2$ hour at 120°) the earth was pressed firmly against the glass with a sterile iron wire, so as to prevent the formation of an open fissure here (see above at p. 84). Finally the edge of the plug and the opening with the glass tube at the bottom of the plug were tightened with melted paraffin. The whole apparatus was placed in a simple wooden frame (*b*) so that it could easily be handled.

Some days before the experiments were started the surface of the soil was inoculated with a certain number of algal germs in 3 drops of liquid, or with a mixture of numerous species of algae (number of germs unknown) likewise in 3 drops of water. Into some of the glasses were put a couple of earthworms which had been cleansed as far as possible by being placed for some days between moist filter papers which were frequently changed. They had then gradually filled their intestine with this substance instead of with earth and must be supposed to be tolerably well cleansed inside as well. After inoculation the glass tubes were kept in a dark cupboard except when the experiments were carried on.

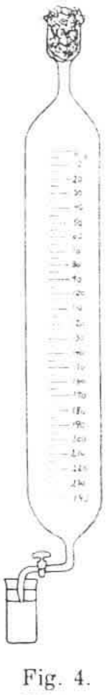

Fig. 4. Water dripper.

A dripping apparatus as shown in Fig. 4 was used for these experiments. It consists of a glass container, about 20 cm long, 4 cm in diameter, with a dividing line for every 5 c.c. At the top the container tapers to a tube provided at the upper end with an expansion designed for a cotton-wool plug. At the bottom the container likewise tapers to a tube with a glass cock and a cork plug to be fitted with a test tube with alcohol.

The container was filled with boiled, filtered tap-water, and was closed with a cotton-wool plug at the top, while the tube and the cock at the bottom were wrapped up in paper, the whole being then sterilised.

This apparatus may be used for dripping water on to the surface of the soil in the glass tubes with an intensity corresponding to that of a heavy thunder shower (at most 1 mm per minute). In order to make the drops strike the earth with a certain force they were dropped from a height of $^1/_2$ m. It was difficult to let them drop from a greater height since it was then not easy to make them hit the surface. As long as dripping took place the glass tube was kept in constant motion, so that the drops were as far as possible evenly distributed over the surface without falling right against the glass wall since this would involve the risk of the algae slipping down through interstices along the latter, even though such had as far as possible been removed.

After a suitable amount of water had been dripped into the glass tube, the dripping was stopped; the water then sank into the soil. After some time some of it will have run right through the layer of soil and can be drawn off through the glass tube at the bottom into the sterile test tube. As a rule 5 c.c. of water were collected (sometimes a smaller amount), which were poured into a sterile Petri dish and mixed with sterile melted agar with Bristol's nutrient medium. After hardening the Petri dishes were exposed to the light, and the algal colonies growing up then showed how many algal germs had passed with the water through the column of earth.

For checking the experiments glass tubes of the usual dimensions were used, which were closed at the bottom by a paraffined cork plug and covered at the top with a glass dish. The tubes were filled with wellmoistened earth and sterilised. On the surface they were inoculated in the same way as the tubes designed for seepage and were placed in the same cupboard with these, but no water was dropped into them. After the seepage experiments had been finished, 5 c.c. of soil were taken from the bottom of the control tubes in sterile glasses, and the water content of the soil of the tube was determined. The soil sample was diluted in 14 tubes in a liquid medium in the usual way, and 1 c.c. of dilution No. 1 was diluted in agar. In no case did these dilutions show any growth of algae.

Experiments with *Pleurochloris magna.*

From previous observations (1932 I, p. 405) I have learned that *Pleurochloris magna* forms zoospores when kept 48 hours in the dark. After the inoculation of the glasses I left them for some days in the dark, and it is then probable that zoospores were formed in quantity, and that the cells that were carried down into the soil in the dripping experiments were chiefly such. On account of their small size the zoospores will presumably more readily be carried downwards than the vegetative cells. The latter are round, quite devoid of mucilage envelopes and so not apt to adhere together in large cell aggregates, but will easily be separated from each other by the falling drops. It must therefore be supposed that this kind of cell, too, will comparatively readily be carried down into the soil by the rain water.

4 glass tubes were inoculated with *Pleurochloris:* No. 1 and No. 2 with earthworms, No. 3 and No. 4 without earthworms; further 2 control tubes: No. 5 with, No. 6 without earthworms. For the

details the reader is referred to the subjoined tables of the experiments.

It turned out that already in the first dripping experiment it was possible to carry algae through the soil in all 4 tubes. In the tubes containing earthworms, which had burrowed from the surface right down to the sandy layer at the bottom, a very large number of algal germs was carried through, especially in the first two experiments. It is not improbable that this is due to the activity of the earthworms. In the later experiments with the same tubes the number of algal germs was less, which is perhaps due to the smaller amount of water in the later experiments. But on this point the experiments are not unambiguous, tube 4 occupying a special position owing to the fact that the number of algal germs was larger in the second and third experiments than in the first.

The control experiments with glass tubes 5 and 6 showed that the algae did not move from the surface down to the bottom without the aid of the seeping water, and that even the presence of earthworms made no difference.

Table 30. Glass tube No. 1. Depth of layer of soil 17 cm. Depth of sand and stone layer 3 cm. Inoculated $^{6}/_{5}$ 32 with 426,000 cells of *Pleurochloris* in 3 drops. 2 earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{10}/_{5}$	20 mm	0.9	5 ccm	228
2	$^{13}/_{5}$	7.5 -	0.8	5 -	42

Table 31. Glass tube No. 2. Depth of layer of soil 13 cm. Depth of sand and stone layer 4 cm. Inoculated $^{6}/_{5}$ 32 with 426,000 cells of *Pleurochloris* in 3 drops. 2 earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{13}/_{5}$	11 mm	0.6	5 ccm	1056
2	$^{14}/_{5}$	2.5 -	0.6	$2^{1}/_{2}$ -	69
3	$^{14}/_{5}$	2.5 -	1.2	5 -	30
4	$^{14}/_{5}$	2.5 -	0.8	5 -	59

Table 32. Glass tube No. 3. Depth of layer of soil 12 cm. Depth of sand and stone layer 3.5 cm. Inoculated with 426,000 cells of *Pleurochloris* in 3 drops. No earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{17}/_{5}$	10 mm	0.7	2.5 ccm	113
2	$^{17}/_{5}$	2.5 -	0.6	5 -	14.4
3	$^{17}/_{5}$	2.5 -	0.4	5 -	7.2
4	$^{17}/_{5}$	2.5 -	0.3	5 -	9.6

Table 33. Glass tube No. 4. Depth of layer of soil 14 cm; depth of sand and stone layer 3 cm. Inoculated $^{6}/_{5}$ 32 with 426,000 cells of *Pleurochloris* in 3 drops. No earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{17}/_{5}$	10 mm	0.5	1.5 mm	40
2	$^{18}/_{5}$	5 -	0.6	5 -	86.4
3	$^{19}/_{5}$	5 -	0.8	5 -	75.6
4	$^{19}/_{5}$	2.5 -	0.6	5 -	33.6

Control.

Glass tube No. 5. Inoculated $^{6}/_{5}$ 32 with 426,000 cells of *Pleurochloris* in 3 drops. 2 earthworms. Left in the dark.

5 c.c. of soil from the bottom diluted $^{11}/_{6}$ 32 with liquid in 14 tubes and 1 c.c. of dilution No. 1 in agar.

At the bottom the soil contained 25.3 p.c. of water. No algae developed.

Glass tube No. 6. Inoculated $^{6}/_{5}$ 32 with 426,000 cells of *Pleurochloris* in 3 drops; no earthworms; kept in the dark.

5 c.c. of soil from the bottom diluted $^{11}/_{6}$ 32 with liquid in 14 tubes, and 1 c.c. of dilution No. 1 in agar.

At the bottom the soil contained 24.6 p.c. of water. No algae developed.

Experiments with *Nitzschia Kützingiana* var. *terrestris*.

Nitzschia Kützingiana Hilse var. *terrestris* was described by the present writer in 1932 I, p. 407. It is one of the smallest known forms of the genus and like all Nitzschiae it has the power of active movement. It has also a certain tendency to adhere to the particles of earth, so that it might be expected at the outset that it could not so easily be washed down through the soil with a stream of water. The number of cells sown was smaller, too, than in the case of *Pleurochloris*, and finally a later investigation showed that hardly all the cells sown were viable. This control experiment was carried out as follows. 5 c.c. of soil from half the surface in tubes 7 and 9 were scraped off and partly diluted in the usual way in 14 test tubes, and partly used for an agar plate with 1 c.c. of dilution No. 1. In glass tube 7 the liquid dilution revealed 1000 *Nitzschia* germs in the 5 c.c. of soil, or 2000 germs on the whole surface of he tube; in the agardilution no colonies appeared. From glass tube No. 9 1000 germs were found in the liquid dilution, or 2000 germs on the whole surface; 5 colonies appeared in the agar dilution, which makes 2500 germs in 5 c.c., or 5000 germs on the whole surface, thus all in all much lower figures than the 168,000 cells originally counted (see tables 34—37). Even if some of the cells must have been carried down into the soil in the experiment, many of the cells sown must be supposed to have been dead, a fact which is not always easy to ascertain by means of a simple microscopical examination, amongst other things because the counting must take place at a rather low magnification.

In the first series of dripping experiments, in which 4 times 5 c.c. of water were drawn off from each glass tube, it turned out that no *Nitzschia* germs had passed through the soil. I then made another series of dripping experiments, and from tubes Nos. 7 and 8 in which there were earthworms, I was able to get germs through the soil, though in small numbers. Through tubes Nos. 9 and 10, where no earthworms were present, no germs came through the soil in the second experiment either. Hence these experiments would seem to indicate that the earthworms by their burrowing in the soil will facilitate the carrying down of algal germs through water seepage. Further it is probable that Diatoms are not so easily carried downward as green algae, at any rate certain species devoid of mucilage envelopes.

In the control experiments with glass tubes 11 and 12, with and without earthworms respectively, no *Nitzschia* germs could be shown to be present at the bottom of the glass tube.

Table 34. Glass tube No. 7. Depth of layer of soil 13 cm; depth of sand and stone layer 3 cm. Inoculated $^{6}/_{5}$ 32 with 168,000 cells of *Nitzschia* in 3 drops. 2 earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{19}/_{5}$ 32	7,5 mm	0.6	5 ccm	0
2	$^{20}/_{5}$ -	5 -	1.0	5 -	0
3	$^{20}/_{5}$ -	0 -	—	5 -	0
4	$^{8}/_{6}$ -	10 -	0.4	5 -	0.4
5	$^{8}/_{6}$ -	0 -	—	5 -	0
6	$^{8}/_{6}$ -	0 -	—	5 -	0.2

Table 35. Glass tube No. 8. Depth of layer of soil 15 cm; depth of sand and stone layer 3 cm. Inoculated $^{6}/_{5}$ with 168,000 cells of *Nitzschia* in 3 drops. 2 earthworms.

Fxperiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{18}/_{5}$ 32	17.5 mm	0.9	6 ccm	0
2	$^{19}/_{5}$ -	5 -	0.7	3 -	0
3	$^{19}/_{5}$ -	2.5 -	0.8	5 -	0
4	$^{19}/_{5}$ -	2.5 -	0.8	5 -	0
5	$^{7}/_{6}$ -	10 -	0.6	5 -	0.6
6	$^{7}/_{6}$ -	0 -	—	5 -	0
7	$^{7}/_{6}$ -	0 -	—	5 -	0.2

Table 36. Glass tube No. 9. Depth of layer of soil 17 cm; depth of sand and stone layer 3 cm. Inoculated $^{6}/_{5}$ 32 with 168,000 cells of *Nitzschia* in 3 drops. No earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{20}/_{5}$ 32	12.5 mm	0.5	5 ccm	0
2	$^{20}/_{5}$ -	2.5 -	0.5	3 -	0
3	$^{20}/_{5}$ -	2.5 -	0.5	5.5 -	0
4	$^{8}/_{6}$ -	10 -	0.4	5 -	0
5	$^{8}/_{6}$ -	0 -	—	3 -	0

Table 37. Glass tube No. 10. Depth of layer of soil 12 cm; depth of sand and stone layer 3.5 cm. Inoculated $^{6}/_{5}$ 32 with 168,000 cells of *Nitzschia* in 3 drops. No earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c.c.
1	$^{27}/_{5}$ 32	12.5 mm	0.6	5 ccm	0
2	$^{27}/_{5}$ -	0 -	—	2 -	0
3	$^{27}/_{5}$ -	2.5 -	0.5	5 -	0
4	$^{27}/_{5}$ -	2.5 -	0.8	3 -	0
5	$^{7}/_{6}$ -	10 -	0.7	5 -	0
6	$^{7}/_{6}$ -	0 -	—	5 -	0
7	$^{7}/_{6}$ -	0 -	—	5 -	0

Control.

Glass tube No. 11. Inoculated $^{6}/_{5}$ 32 with 168,000 cells of *Nitzschia* in 3 drops of liquid; 1 earthworm; kept in the dark.

$^{23}/_{6}$ 32. 5 c.c. of soil from the bottom diluted in liquid in 14 tubes; 1 c.c. of dilution No. 1 diluted with agar.

The earth at the bottom contained 21.9 p.c. of water. No algae developed in the dilutions.

Control.

Glass tube No. 12. Inoculated $^{6}/_{5}$ 32 with 168,000 cells of *Nitzschia* in 3 drops of liquid; no earthworms; kept in the dark.

$^{23}/_{6}$ 32. 5 c.c. of soil from the bottom diluted in liquid in 14 tubes; 1 c.c. of dilution No. 1 diluted in agar. The earth at the bottom contained 23.9 p.c. of water.

No algae developed in the dilutions.

Experiments with a mixed culture of various soil algae.

For inoculation of tubes 13—15 I employed a culture of 5 c.c. of garden soil + 3 c.c. of water in a Freudenreich flask after it had been exposed to the light for some length of time. The soil was suspended in about 10 c.c. of water, and 3 drops at a time were taken from the mixture, which were added to the surface of the soil in each glass tube. The culture contained numerous species of Chlorophyceae, Diatoms, and Cyanophyceae. It could not be established how many germs were present in each drop, since the greater part were filamentous algae or species whose cells were imbedded in mucilage. It can merely be said that a very great number of germs were present. As will appear from the subjoined tables for tubes 13 and 14, germs of Chlorophyceae (including Heterokontae) as well as of Diatoms and Cyanophyceae passed through the soil in the dripping experiments, already in the first experiment. It is worth noting that several colonies of a Hormidium form appeared on the agar plate, from which it will be seen that filamentous algae, too, can be washed down through the soil. However, the number of germs of green algae was particularly high, as much as 88.3 per c.c. of water drawn off. In glass tube No. 13 only some few Diatoms passed through in a single experiment, whereas up to 13.6 Diatom germs per c.c. of water drawn off passed through tube No. 14. The species of Diatom which appeared on the agar plate was found to be *Navicula cryptocephala* var. *veneta*, and it was further ascertained that this was the dominant species in the starting material.

The Cyanophycea that passed through the soil proved to be a Nostoc species, and it must then no doubt be its hormogonia which have been carried along with the water. On the agar plate it showed a very lively formation of hormogonia, and the individual hormogonia passed out through the agar from the centre of the colony and formed small new colonies. The number of *Nostoc* germs was rather small, at most 2.7 per c.c. of water drawn off. In the control tube No. 15 no algae could be shown to be present in the soil at the bottom of the tube.

Table 38. Glass tube No. 13. Depth of layer of soil 13 cm; depth of sand and stone layer 3 cm.
Inoculated $^{6}/_{5}$ 32 with a rich culture of algae; 3 drops; no earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm pr. min.	Amount of water drawn off	Germs per c. c.	Chloroph. per c. c.	Diat. per c. c.	Nostoc per c. c.
1	$^{27}/_{5}$ 32	10 mm	0.8	3 c. c.	85	84	0	1
2	$^{27}/_{5}$ -	2.5 -	0.4	5 -	35.4	34.8	0.2	0.4
3	$^{28}/_{5}$ -	2.5 -	0.5	2.5 -	36	36	0	0
4	$^{28}/_{5}$ -	2.5 -	0.8	5 -	48	48	0	0

Table 39. Glass tube No. 14. Depth of layer of soil 13 cm; sand and stone layer 3 cm.
Inoculated $^{6}/_{5}$ 32 with a rich culture of algae; 3 drops; no earthworms.

Experiment	Date	Amount of water dropped in	Intensity mm per min.	Amount of water drawn off	Germs per c. c.	Chloroph. per c. c.	Diat. per c. c.	Nostoc. per c. c.
1	$^{28}/_{5}$ 32	10 mm	1.0	2.5 ccm	44.8	43.2	1.2	0.4
2	$^{28}/_{5}$ -	5 -	0.8	5 -	47.4	43.2	3.2	1.0
3	$^{28}/_{5}$ -	2.5 -	0.5	5 -	76.8	64.8	10.8	1.2
4	$^{28}/_{5}$ -	0 -	—	3 -	88.3	72.0	13.6	2.7

Control.

Glass tube No. 15. Inoculated $^{6}/_{5}$ 32 with a rich culture of algae; 3 drops; no earthworms; kept in the dark.

$^{17}/_{6}$ 32. 5 c.c. of soil from the bottom diluted with liquid in 14 tubes; 1 c.c. of dilution No. 1 diluted in agar.

The earth at the bottom contained 23.6 p.c. of water.

No algae developed in the dilutions.

c. The Importance of Earthworms in the Carrying down of Algae into the soil.

The experiments described in the previous section on the effect on algae of water seepage also furnish hints as to the activity of earthworms in the way of carrying algae into the soil, even though they are not satisfactory in this respect. In nature earthworms are continually passing up and down through the soil (down to depths of more than 1 m (BORNEBUSCH 1930, p. 89)), and burrow in it in all directions. They take vegetable matter from the surface, draw it into the earth and swallow it. The excrements are generally voided at the surface. It would seem natural to assume that during this activity the worms would come to carry algal germs with them into the lower layers of the soil. Partly it may be supposed that algal germs may adhere to the outside of them, and partly they may be inside them, in the contents of the intestine. It is possible, however, that the slimy surface of the worm will make it difficult for the germs to adhere to them, and that not many germs are carried downwards in this manner. Whether algal germs will survive the passage through the intestine of an earthworm is not known with certainty, and the greater part of the excrements are, as far as is known, discharged at the surface. Here we shall merely refer the reader to the fact that FRANCÉ (1913, p. 87) found living *Navicula mutica* in the intestinal content of a live earthworm.

On the other hand, vegetable matter drawn down into the soil by the worms may easily be imagined to carry algae with it from the surface.

The experiments, especially the control experiments with tubes 5 and 11, would, indeed, seem to show that earthworms do not carry algal germs directly into the soil with them.

On the other hand, it is probable that indirectly earthworms contribute largely to carrying down the algae, viz. by their numerous burrows in the earth, and by their action in making the soil loose. This appears pretty plainly from the experiments. A much larger number of Pleurochloris germs passed through tubes 1 and 2 which contained earthworms than through tubes 3 and 4 which contained no worms. In the experiments with *Nitzschia* it turned out that it was only possible to make algal germs pass through the glass tubes containing earthworms (Nos. 7 and 8), while no Nitzschia germs passed through the tubes that did not contain worms.

We then arrive at the conclusion that earthworms probably to a slight degree only carry algae down themselves, but by burrowing in the earth and loosening it they make it easy for seeping water to wash down the algae.

3. The Soil Algae in Relation to their Water Supply.

a. The Conditions of Moisture in the Soil.

In our climate there will always be a sufficiency of water for the algae in the lower layers of the soil. Here the air will always be saturated with water (RAMANN 1911, p. 390), so there will hardly be any risk of desiccation. Even through very long periods of drought the soil will only be totally desiccated to a very slight depth, and owing to the formation of dew in the night, partly from the atmosphere, partly from the lower layers, desiccation will only reach its highest intensity during the daytime, whereas it will always be somewhat less at night (LEICK 1933, p. 410, RAMANN 1911, p. 382).

It is merely for the algae on the surface itself that desiccation may reach high degrees, when drought in connection with strong insolation asserts itself; but the percentage of water will undoubtedly vary greatly for each day, since precisely the surface will derive most advantage from the formation of dew.

When the top layer of the soil is cooled in the night by radiation, the temperature of the air above it may reach the dew-point, so that a further cooling will cause a formation of dew on the surface. At the same time the moisture from the lower layers, which are now warmer than the surface, may become condensed in the latter. The water thus formed will in the daytime partly be again given off to the atmosphere, partly also to the lower layers which are now the coldest. Since the air contained in the soil is always saturated with water, even small differences in temperature may involve a very considerable redistribution of the water in the soil (RAMANN l. c., LEICK l. c.).

The formation of dew on the leaves of higher plants (trees and shrubs) may also, at any rate in certain climates, reach such a degree that the water drips from them on to the soil (LEICK 1933, p. 410).

The deposition of dew on the surface itself will undoubtedly chiefly occur in places where it is not covered by any closed vegetation of higher plants (LEICK 1933, p. 413). Here perhaps we meet with something which will contribute to explain a fact that I have repeatedly mentioned (1915, p. 301, 1932 I, p. 12), the fact that on soil under a dense covering of plants, as e. g. dense heather, grass, or a deep layer of withered leaves, the soil alga flora will as a rule be poor. It must be supposed, however, that conditions of illumination, as I have previously pointed out, are of most importance.

The chief thing to the algae is not, presumably, the average water percentage of the surface layer, but rather the absolute minimal values. The available figures in this respect do not seem particularly low. SCHRÖDER (1886, p. 29) mentions that he determined the water content of exposed clayey soil mixed with sand in the afternoon after a period of unusual drought. The layer of soil scraped off was not 2 cm deep. In this there were still 6 p. c. of water. I myself have made similar determinations (1915, p. 275) and tried to collect even more superficial samples, only going down to a depth of $^1/_2$—1 cm. In garden soil rich in humus I found as little as 5.2 p. c. of water after a not very intensive drought, and there is no doubt that the top mm where the soil algae are chiefly found may at times contain a considerably smaller amount of water. Still the soil algae will probably always be better off with regard to water than the eu-aërial algae, which are reduced to the exclusive use of atmospheric water. For these the change from moist to dry periods will occur much more rapidly, so that they will not have time to protect themselves against desiccation by the formation of resting stages. For the soil algae desiccation will cover a longer period, and many of them will therefore have time to form more or less pronounced resting stages to counteract the unfavourable external conditions.

b. The Adaptation of the Soil Algae to resist Desiccation.

Special resting stages are not known in the soil Diatoms (only in plankton forms). It has been supposed that forms with "innere Schalen" (previously often called craticular forms) were a kind of resting cells (SCHRÖDER 1886, p. 27, where literature is also quoted on other phases of Diatoms which have been regarded as resting stages). As regards these forms GEITLER (1927) has recently put forward the suggestion that the occurrence was due to want of nourishment. Since Diatoms with double valves are not unusual in salines with highly varying salinity, it has also been conjectured that their formation was connected with this variation (O. MÜLLER 1899, p. 282, KRASSKE 1927, p. 270). But it is not probable that these formations can be interpreted as resting stages, the purpose of which should be to enable the species to survive periods of drought (BOYE PETERSEN 1928 I, p. 328). More probably their occurrence is a direct consequence of the highly varying concentration of the liquid in the soil.

It is quite another thing that the cell content of the soil Diatoms may become changed during desiccation in a way that may be supposed to contribute to make the cells resistent to desiccation. Thus in Diatom cells from dry soil samples large masses of an oleaginous substance are often seen which may almost cover the chromatophores. This very much resembles the changes in the cell content of various Chlorophyceae of which it has been shown experimentally that precisely the cells filled with granulations are the most resistent (FRITSCH and HAINES 1923, p. 724; PIERCY 1917, p. 533).

The first to undertake experiments on the capability of Diatoms to resist desiccation was SCHRÖDER (1886, p. 28). He left a number of small flowerpots to stand with soil containing numerous Diatoms, so that the earth was gradually dried up. At certain intervals he examined one of the pots to see whether the Diatoms were alive and how much water the earth now contained. It turned out that when the earth contained 9.05 p.c. of water, the Diatoms were dead. In this connection I have previously pointed out (BOYE PETERSEN 1915, p. 274) that the Diatoms used by SCHRÖDER appear to have been hydrophilous species, not terrestrial, and it cannot, therefore, be taken for granted that the latter cannot resist a more intense desiccation than his experiments show. As a matter of fact I found that *Hantzschia amphioxys* and *Navicula mutica* were in full vegetative activity in soil with only 5.2 p.c. of water (l. c. p. 275).

The Cyanophyceae often possess an equipment which must be regarded as a means of protection against desiccation, viz. their more or less well developed mucous sheaths, which are not rarely of a very firm consistency and provided with differently coloured pigments. These mucous sheaths must be able to aid in retaining water during droughts and also in the absorption of water when moisture reappears (SCHRÖDER 1886, p. 31; FRITSCH 1922 II, p. 232).

But it also turns out that even apparently unprotected cells of *Oscillatoria* often resist a very protracted and intensive desiccation without suffering any injury (GLADE 1914, p. 334, SCHRÖDER 1886, p. 32), a circumstance which FRITSCH ascribes to the nature of their cell contents without distinct vacuoles and filled with granulations which, it is true, are of a different character to those of the Chlorophyceae examined by him. In addition, many Cyanophyceae are able to form special resting spores, which are very resistent to desiccation. Experiments in this field were made by GLADE (1914, p. 334) who found that the spores of *Cylindrospermum licheniforme*, *C. muscicola*, and *Nostoc* sp. could resist a protracted, intense desiccation (in an exsiccator), whereas the vegetative cells died from the treatment (cf. also SCHRÖDER 1886, p. 32). Very little is known about the conditions for the formation of resting stages in Cyanophyceae; but it has been conjectured (GEITLER 1930, p. 37), that the cause might be want of nourishment, desiccation, or other external factors. GLADE indeed found

(1914, p. 329) that the *Cylindrospermum* species formed resting spores when there is a lack of nutrient substances in the cultures, amongst other things when N or P is absent from the medium.

Among the Chlorophyceae not a few are able to withstand complete desiccation without taking any harm, though the cells have apparently no protection in the shape of thickening of the walls, mucous sheaths or the like. This applies especially to many eu-aërial algae, *Apatococcus*, *Pleurococcus*, *Trebouxia*, and others, which grow on prominent objects.

Certain terrestrial algae may possess the same properties, though as a rule in less marked degree, as *Prasiola* and *Hormidium flaccidum*.

FRITSCH with various co-workers undertook some experiments for the purpose of finding out to what this power of resisting desiccation was due.

He found that the water content (FRITSCH 1922 I, p. 5) of air-dry algae such as *Pleurococcus*, *Prasiola crispa* (in the *Hormidium* form), and *Zygnema ericetorum* fluctuated greatly with the degree of humidity in the air, more than the water content of non-living material. Air-dry cells of *Prasiola* and *Pleurococcus* and to a less degree of *Zygnema* will, when heated to 100°, give off a good deal of water which cannot later be regained by absorption from the air; thus they possess a special power of retaining a certain amount of water (FRITSCH 1922 I, p. 15). He believes that this power may be due to the high concentration of the cell-sap which does not occur, or occurs only in very slight amount, in vacuoles, but is more probably imbibed in the protoplasm. FRITSCH (l. c.) also ascribes to their aërial life the fact that these algae, even if offered plenty of water, are only able to absorb comparatively little of it, since it follows that small amounts of water may after desiccation again enable them to continue their interrupted growth and other vegetative functions. This power is most marked in *Pleurococcus*, less in *Prasiola* and *Zygnema*.

The cell contents of many algae under certain circumstances, e. g. slow desiccation, exhibit granulations which may gradually collect in large masses of an oleaginous appearance; the phenomenon has been mentioned by a long series of authors (see PIERCY 1917, p. 525), who by no means agree about the chemical nature of the secreted substance (FRITSCH and HAINES 1923, p. 721; PIERCY 1917, p. 526), nor about its significance for the algae.

FRITSCH (1916, p. 143, 148) connects the formation of these drops or granules with the capacity of *Zygnema ericetorum* to withstand desiccation, and PIERCY (1917, p. 527) interprets them in the same way for *Hormidium flaccidum*. Her experiments would also seem to show that cells with granulations are more resistent than cells without granulations. FRITSCH and HAINES (1923) find that of the individual cells in a filament of *Prasiola* or in the same growth of *Pleurococcus* or *Cystococcus* (= *Trebouxia*), some prove very easy to plasmolyse; for others more vigorous means are required, and others again can only be plasmolysed by solutions of a very strong osmotic effect. Now these cells mostly turn out to be precisely those which have a highly granular protoplasm. It is hardly probable, however, that these granules in themselves are the cause of the capacity which the cells possess to resist desiccation; their occurrence must more probably be regarded as a concomitant of something which is the real cause. (PIERCY 1917, p. 533).

By means of centrifugalising experiments and by employing dark-ground illumination FRITSCH and HAINES (l. c. pp. 718, 720) have shown that the algal cells most resistent to desiccation in contrast to the cells of hydrophytic algae possess a very viscous protoplasm, in which Brownian movements cannot be observed. They think that this character of the protoplasm is the cause of the power possessed by the cells of retaining water and so surviving periods of drought.

Finally FRAYMOUTH has shown (1928) that aërial algae, especially *Prasiola crispa* and *Trebouxia* have virtually no respiration in the dry state, while this process becomes increasingly brisk when the moisture increases. In *Trebouxia* it is again checked when the cells are covered with a water film, which evidently renders respiration difficult. After a long period of drought a supply of moisture will almost immediately be able to start respiration again, from which it must be inferred that during drought these algae are merely at rest for the time and can therefore at once be awakened to renewed animation when moisture is supplied.

In contrast herewith the resting cells mentioned by KLEBS (1896, p. 340), which are formed in a number of soil algae such as *Hormidium nitens* and the *Bumilleria* species, (pp. 388, 392), will no doubt only after having been for some time in a moist environment be able to continue their vegetative functions with unabated vigour. These cells are more radically changed, they are not only filled with granules of fatty substances, but their walls have also become somewhat thickened.

It appears then that the eu-aërial algae are constantly in a condition in which the cells will survive a very sudden desiccation and likewise desiccation during a protracted period, and this is due to the peculiar nature of their protoplasm.

Hydrophytic algae will never in the vegetative condition be able to survive a desiccation; in these only resting spores especially adapted for the purpose (often zygospores or oospores) can withstand desiccation.

The soil algae occupy an intermediate position. Upon rapid desiccation all, or at any rate many, cells will die. During slow desiccation most of them are able to change the nature of their cells in such a way that they can resist desiccation. This applies especially to the Chlorophyceae, in which more or less pronounced resting cells are found. In *Prasiola* the resistent cells are not very different from the non-resistent: in *Hormidium* and *Bumilleria* the difference is more marked, and in *Vaucheria* and *Botrydium* it is only the most pronounced resting cells (oospores and hypnocysts) that are able to withstand intensive desiccation.

The Diatoms seem in the main to behave like *Hormidium;* among the Cyanophyceae many Oscillatoriaceae exhibit a similar behaviour, while *Nostoc* and especially *Cylindrospermum* can only manage by means of specially adapted resting spores.

These facts also apply to BRISTOL ROACH's experiments on rapid desiccation of a soil sample, which have previously been mentioned (p. 28).

c. The Capacity of Soil Algae to survive Desiccation.

Miss BRISTOL's statement in 1919 that she had found great numbers of living algal germs in soil samples from 26 to 73 years old created a great sensation. These samples had been kept in bottles with lead covers, so they were not completely desiccated but contained from 3 to 10 p.c. of water. Portions were kept for 3 years in tubes plugged with cotton-wool, and during that time there was evaporation of water from them, so that finally they all contained about 3 p.c. of water. These soil samples were added to a solution of mineral salts and the cultures exposed to the light. A large number of species of Cyanophyceae, Chlorophyceae, and one species of Diatom (*Nitzschia Palea*) then developed in the cultures.

The samples had not been taken in a sterile way, and not until later, when Miss Bristol received them for examination, was a sterile treatment carried through. Bristol, also, was not able to clear up which algae will best survive being kept so long; Diatoms however, seemed to be the least resistent, since she only found one species of these.

I have therefore thought it appropriate to repeat the experiment in a somewhat different manner. I wished especially 1) to make the experiment with soil samples treated in an absolutely sterile way from the beginning; 2) to let these dry up entirely at room temperature and in the dark; 3) to ascertain at suitable intervals during the experimental period the water content of one of the samples as also what algal species and how many germs it contained. I thought it possible to establish that germs of certain species would totally perish, while others would prove more resistent. In the mixed cultures I set up to begin with, however, conditions proved so unstable that there seemed no probability of attaining this object. I therefore adopted a dilution method in order to ascertain the approximate number of living algal germs in the samples. During the experimental period given (3 years) it was not possible to show that any decrease in the number of germs had taken place. It is possible that a greater or smaller number of germs have perished during the first time when the number of germs was not ascertained; we shall return to this point later on. But the experiment shows that the germs which have survived the first desiccation are evidently able to survive very long in the desiccated state.

The experimental technique was as follows:

From a place in the Botanical Garden of Copenhagen where the earth is not dug I collected, on $^8/_3$ 30, 12 samples of soil of about 20 c.c. in sterile test tubes with cotton-wool plugs. They were placed in a dark cupboard in a room which was kept at constant ordinary room temperature (c. 20° C.) by means of central heating. Since nothing was done to produce moisture, the air was always very dry, at any rate during that part of the year when the room was heated. During summer the degree of humidity in the air might evidently increase somewhat.

On $^8/_3$ 30 the water content of sample No. 1 was found to be

17.5 p.c. A small portion of the sample (about $^1/_2$ c.c.) was transferred to some BRISTOL's liquid medium (half-and-half) in a Freudenreich flask. The rest of the sample was left in the original tube and moistened with sterile water. Both tubes were exposed to the light, and algae grew up in them. On account of the relatively large cotton-wool plug the last tubes dried up rapidly again. In these especially certain Cyanophyceae and Diatoms appeared.

The succeeding samples, No. 2 ($^7/_4$ 30), No. 3 ($^8/_5$ 30), No. 4 ($^{16}/_9$ 30) weretreated in the same way as No. 1. There was a rapid decrease in the water content of the samples, so that already sample No. 2 only contained 1.5 p.c. of water, No. 3: 1.37 p.c., and No. 4: 1.4 p.c.

Sample No. 5 was inoculated ($^{10}/_{11}$ 30, 1.4 p.c. of water) in the same way as the preceding ones; but in addition a sample of about 5 c.c. was taken from it and diluted in 14 test tubes in the way indicated above. Judging from this dilution it contained about 100,000 algal germs per c.c. of soil.

Sample No. 6 was treated like No. 5 ($^{11}/_3$ 31), and then contained 1.35 p.c. of water. The dilution revealed about 20,000 germs per c.c. of soil.

Sample No. 7 was treated like the two preceding samples ($^{29}/_6$ 31). The percentage of water was then found to be considerably higher than in the two preceding samples, viz. 4.3 p.c. However, perhaps this was due to erroneous weighing. The dilution gave a result of about 100,000 germs per c.c. of soil. I had also set up a dilution on agar of this sample, and the counting of the colonies on the agar plate gave as a result 71,000 germs per c.c. of soil. Owing to the unusually high percentage of water in sample No. 7, I took out sample No. 8 for examination some few days later ($^{11}/_7$ 31). The water percentage of this sample was found to be 1.71 p.c., i. e. somewhat higher than in the preceding samples (except No. 7), still not higher than might be supposed to be due to the fact that the air in the room where the samples were kept is more humid in summer than in winter when it is artificially heated. Sample No. 8 was treated exactly like sample No. 7, and the dilution in test tubes gave a result of 20,000 germs per c.c. of soil; the dilution in agar showed 27,000 germs per c.c. of soil.

As will appear from Table 40, the following samples were examined at increasing intervals of time; their water percentage and content of algal germs remained almost unaltered until $^3/_3$ 33, when the last sample was examined, 3 years after the first.

The numbers of germs found in these desiccated samples may be compared with the figures which I have found for surface soil from the same locality in freshly-collected samples, viz.

Table 2,	p. 36	$^{9}/_{4}$	> 200,000	germs	per c.c.	of soil
— 3,	p. 36	$^{19}/_{10}$	20,000	—	—	—
— 5,	p. 38	$^{5}/_{4}$	3,000,000	—	—	—
— 27,	p. 67	$^{27}/_{10}$	95,000	—	—	—
— —	—	—	150,000	—	—	—

It will then be seen that in April there were as a rule many more germs present than I found in any of the dry samples; in the autumn, on the other hand, there were about the same number as in these. This would seem to show that during the desiccation in the first months a number of germs perished in the desiccated samples.

Table 40. Desiccated soil; samples collected $^{8}/_{3}$ 30.

No.	Date	Time left dry in months	Water percentage	No. of species	No. of germs with dilution in liquid	No. of germs with dilution in agar
1	$^{8}/_{3}$ 30	0	17.5	14	—	—
2	$^{7}/_{4}$ -	1	1.5	7	—	—
3	$^{8}/_{5}$ -	2	1.37	8	—	—
4	$^{16}/_{9}$ -	6	1.4	14	—	—
5	$^{10}/_{11}$ -	8	1.4	19	100,000	—
6	$^{11}/_{3}$ 31	12	1.35	11	20.000	—
7	$^{29}/_{6}$ -	16	(4.3?)	14	100,000	71,000
8	$^{11}/_{7}$ -	16	1.71	14	20,000	27,000
9	$^{20}/_{10}$ -	20.5	1.17	21	100,000	74,000
10	$^{8}/_{3}$ 32	24	1.35	17	100,000	68,000
11	$^{9}/_{9}$ -	30	1.5	20	20,000	83,000
12	$^{3}/_{3}$ 33	36	0.9	15	200,000	131,000

Table 41 gives the species of algae found in the various samples. From this it appears very plainly that at any rate the fairly common species of all three groups have survived desiccation for three years. In the case of the Chlorophyceae there would even appear to be more species present in the later samples than in the first ones. This, however, is undoubtedly due to the improved method of cultivation. In a simple culture in a Freudenreich flask ino-

Table 41.

Sample Nos	1	2	3	4	5	6	7	8	9	10	11	12
Months left dry	0	1	2	6	8	12	16	16	20.5	24	30	36
Anabæna sp.	+	+	+	..	..	+	..	+	..	..	+	..
Cylindrospermum sp.	..	..	..	..	..	..	..	..	+	..	..	+
Microcoleus vaginatus	+	+	+	+	+	+	+	..	+	+	+	+
Phormidium foveolarum	..	..	..	..	..	+	..	..	..	..	..	..
Plectonema Battersii	..	..	..	..	..	..	..	..	..	+	..	..
Hantzschia amphioxys	+	+	+	+	+	+	+	+	+	+	+	+
Navicula Atomus	+	..	..	+	+	..	+	+	+	+	+	+
Navicula Borrichii	+	..	..	+	+	..	..	..	+	+	+	..
Navicula cincta v. Heufleri	+	..	..	..	..	..	..	..	..	..	..	..
— gibbula v. terrestris	+	+	..	..	+	..	..	..	+	..	..	..
— mutica f. Cohnii	+	+	..	+	+	+	+	+	+	..	+	+
— — - Goeppertiana	+	..	+	+	+	..	..	..	+	+	+	+
— — - minima	+	..	+	..	..	+	..	..	+	..	+	+
— — - quinquenodis	+	..	..	..	..	+	..	..	+	+	+	..
— — - rhomboidea	..	..	..	..	..	..	..	..	..	+	+	..
Pinnularia borealis	..	+	..	+	..	..	..	..	..	..	+	..
— obscura	+	..	..	..	..	..	..	..	..	..	..	..
Bumilleria exilis	..	..	+	+	+	..	+	+	+	..	+	+
— sicula	..	..	..	..	..	..	..	..	..	..	+	..
Bumilleriopsis brevis	..	..	..	..	..	..	+	+	+	..	..	..
Chlamydomonas sp.	..	..	..	+	+	..	..	+	+	+	+	+
Chlorella botryoides	..	..	..	..	..	+	+	+	+	..	..	..
— vulgaris	..	..	..	+	+	..	+	+	+	..	..	..
Chlorococcum humicola	+	..	+	+	+	+	+	+	+	+	+	+
Dictyococcus minor	..	..	..	+	+	..	+	+	+	+	+	..
Dictyosphaerium minutum	..	..	..	..	+	..	..	..	..	..	..	..
Fernandinella alpina	..	..	..	..	+	..	+	+	+	+	+	+
Geminella terricola	..	..	..	..	..	+	..	..	..	..	..	..
Heterococcus viridis	..	..	..	..	+	..	+	..	+	+	+	+
Hormidium flaccidum	+	+	+	+	+	+	+	..	+	+	+	+
Macrochloris dissecta	..	..	..	+	+	..	..	+	+	+	+	+
Muriella terrestris	..	..	..	..	..	..	+	+	+	+	..	+
Pleurochloris sp.	..	..	..	..	+	..	..	..	..	..	..	..
Stichococcus bacillaris	..	..	..	..	+	..	..	..	..	..	..	..
No. of species	14	7	8	14	19	11	14	14	22	16	20	15

culated with a large amount of soil there will no doubt be a close competition between the numerous species of green algae, and in such a culture it will be impossible to find all the different species that have developed in it. In the dilution cultures competition will partly be done away with, and a survey of the algae growing up will be facilitated. These facts do not apply to Diatoms because they grow well in the Freudenreich flasks and do not thrive better in the dilution cultures.

As a result of the investigation we then arrive at the following facts:

1) During a fairly quick desiccation (covering about 1 month) of a soil sample a not inconsiderable number of algal germs will most likely die; but probably none of the species present will perish entirely.
2) Thereupon, even if the desiccation continues, the number of germs will keep fairly constant for several years, and this applies both to Diatoms, Chlorophyceae, and Cyanophyceae.
3) It further appears that in our climate no period of drought, even of very long duration, will be able to kill off the algal germs in the soil, at any rate not such as are found a short distance below the surface. It is not easy to say what will happen during a long period of drought to the algae inhabiting the surface itself, where in addition to desiccation they are exposed to the heat and light of the sun. Judging from conditions in the cultures, it is probable that green algae will be able to survive even under these conditions, but that especially Diatoms will succumb in great number.

4. The Significance of some other Factors for the Soil Algae.

Even though the conditions of moisture must be supposed to have a quite decisive influence on the whole vegetative activity of the soil algae, other factors, too, may no doubt exert their influence and contribute to their more or less vigorous development.

a. Cold.

It must be taken for granted that low temperatures will contribute to reduce the vital processes of the soil algae, just as they do in other plants, and that frosts must be able to kill the cells.

WISLOUGH (1910, p. 179) has made experiments with *Stichococcus bacillaris* f. *major* on various substrata of agar containing inorganic and organic nutrient substances. He lowered the temperature of the algae to — 21°, and once to — 75° C. It then turned out that the various cultures did not behave in the same way; but it seems that in no case were all the cells in a culture killed. He found that it was principally the young cells that succumbed, while the older cells did better.

KÄRCHER (1931) tried a considerably stronger effect of frost on *Stichococcus bacillaris*. She found living cells of this alga in a culture that had been cooled to —70° C. for 8 days, and to from —183° to —192° C. for 13 hours. She also experimented with *Pediastrum Boryanum* and *Hormidium flaccidum*, which proved to be alive after cooling to —70° C. for 5 hours.

GLADE (1914, p. 335) has investigated the resistance to cold of blue-green algae. He arrived at the result that *dry* spores of *Cylindrospermum licheniforme*, *C. muscicola* and *Nostoc* sp., as well as dry trichomes of *Oscillatoria brevis* showed the greatest resistance. They survived cooling to —80° C. Moist spores of *Cylindrospermum licheniforme* withstood the same degree of cold, whereas the three other algae just mentioned only tolerated cooling to — 16° C. in the moist condition. The vegetative filaments of the *Cylindrospermum* species and *Nostoc* died after cooling for 4 days to from —2° to — 8° C. *Oscillatoria*, therefore, will probably be able to winter in the vegetative state, as observed by FRANCÉ (1913, p. 46); on the other hand, the *Cylindrospermum* species and *Nostoc* will as a rule only be able to survive the winter in the form of spores.

FRANCÉ (1913) has also in several cases found Diatoms in nature in full vegetative activity in hard frozen earth (p. 20). Notably he has often met with *Hantzschia amphioxys*.

Altogether, one arrives at the result that there are hardly any of the soil alga species that can be entirely destroyed by the winter cold. But it is probable that many cells will perish during frost, principally the young ones. Above (p. 106) we have seen that most soil algae have a tendency to deposit drops of fatty substances in their cells; in the higher plants the formation of fat in the cells has been thought to be connected with their capability of tolerating frost (MEZ 1905, LIDFORSS 1907, p. 68); the matter is not quite clear, however; still, there is a possibility that the drops of oil in the cells of the algae may be active against frost. It is not known whether other characteristics of the cell content may co-operate in this respect.

During my studies of the Icelandic alga flora (BOYE PETERSEN 1928 II) I was not able to point out any difference between the algal flora of localities exposed to severe frost and that of the snow patches.

b. Heat.

The temperature of the soil will always be subject to greater variations than that of the air (Warming 1918, p. 96), and this applies particularly to the top layer, where the soil algae chiefly occur. In the lower layers of the soil the variations in temperature will gradually be smoothed out. I do not know how much the temperature may rise in the surface of the soil in our climate. High temperatures in the soil will be due to direct insolation, and the strong illumination will thus act simultaneously with the heat; hence it is difficult to say with certainty whether it is the heat or the light, or both these factors that affect the algae. It is not improbable that Diatoms may be killed off by the sun in the summer. This would seem to be indicated by my own experience as well as that of others, from cultures, e. g. on agar, where the Diatoms show great sensibility to direct sunlight. Chlorophyceae appear to be more resistent in this respect.

On the capability of algae to tolerate strong heat very few experiments are available. Glade alone (1914, p. 338) has made some experiments with various Cyanophyceae. In addition to the duration of the influence of the heat it also proved of importance whether the algae were dry or moist. In the former case resistance was greatest. His results were:

Dry spores of:

Cylindrospermum licheniforme	tolerated	20 minutes at 90°
— *muscicola*		10 — - 95°
Oscillatoria brevis (without spores)		2 — - 100°
Nostoc sp.		

Wet spores of:

Cylindrospermum licheniforme	survived	20 minutes at 80°
— *muscicola*	—	20 — - 80° 10 — - 90°
Oscillatoria brevis (sporeless)	—	20 — - 60°
Nostoc sp.	—	20 — - 70°

Vegetative cells of:

Cylindrospermum licheniforme	survived	20 minutes at 35° 10 — - 40°
— *muscicola*		
Nostoc sp.		

As will be seen, rather high temperatures are required to injure these algae permanently; but it is highly probable that *Cylindrospermum* and *Nostoc* can only keep alive in the shape of spores in places exposed to the sun in the summer time when the temperature no doubt easily rises to 40—50° in the surface of the soil.

c. The acidity of the soil,

which so greatly influences the phanerogam vegetation is also of importance for the soil algae. I have previously pointed out (1915, p. 300) that there is a difference in the composition of the algal vegetation on basic and on acid soil. PUYMALY (1924, p. 33) has somewhat amplified my observations and arrives at the result that Chlorophyceae in general thrive best on basic soil; only some few species, such as *Zygnema ericetorum* and *Mesotaenium chlamydosporum*, are almost exclusively confined to acid soil.

On artificial substrata Diatoms have proved incapable of surviving acid reactions (O. RICHTER 1906, pp. 35 and 76). In accordance herewith I have not been able to find Diatoms on moist heath (1915, p. 284). In the cultures of soil samples from Borris Heath mentioned above (p. 45), where the pH value of the soil was particularly low, no Diatoms appeared either. However, this cannot be shown to be due exclusively to the acidity of the soil, since e. g. in certain highmoors with still lower pH values certain Diatoms develop in quantity (*Pinnularia*, *Eunotia*). Cf. also GRAEBNER 1895.

Even nearly allied forms may behave differently towards culture media of different pH values. Thus MÜNSTER STRÖM (1928) investigated the growth of 4 different *Hormidium* forms at pH 3—8.5. One of the forms grew best at pH 3, another at pH 4.5, and two at pH 5.5.

HOPKINS and WANN (1926) found in culture experiments with an unidentified species of *Chlorella* that the full effect of changes in the pH value of the culture medium were only attained if the iron was simultaneously kept in solution. At higher values of pH (more than 5.7) the iron is apt to be precipitated and the alga will therefore thrive badly, unless by the omission of calcium and the addition of sodium citrate the iron is kept in solution. It then turned out that in that case the alga under investigation grew best at pH 7.5. It was then able to grow at pH values differing as much as from 3.4 to over 7.5. It would seem, then, that within very wide limits the pH value in itself is without any great importance for the growth, but that it is more probably the chemical processes in the substratum consequent upon the degree of acidity which are decisive. This may perhaps serve to explain the contradictory statements occurring in the literature with respect to the relation of the algae to the reaction of the nutrient medium (RICHTER 1911, pp. 99—103). This author, however, thinks that the data warrant the inference that a slightly alkaline reaction is best for most algae.

For the present, then, we know very little about how the soil algae behave in nature, and closer investigations are called for. I shall merely note that RICHTER and ORLOWA (1928) have also studied the relation of the soil algae to the acidity of the soil (see above p. 20).

d. Nutrient substances.

FRANCÉ (1913, pp. 66—84) found that the Edaphon may be used as an indicator of the fertility of the soil. The better the soil, the more micro-organisms did he find in it. However, as usual it is very difficult to gain a clear impression from his work as to the exact importance for the algae of the nature of the soil, since he treats them throughout in conjunction with the protozoa.

On cultivating soil samples (BOYE PETERSEN 1915, p. 282) the present writer found that arable and garden soil are generally richer in Diatom species than other soils. This may be supposed to be due to various causes; but it is probable that the greater content of nourishment in the soil has been of importance.

In Iceland I was strongly impressed with the fact that the alga vegetation was most clearly characterised by the greater or less content of manure in the soil (BOYE PETERSEN 1928 I, p. 353). When the amount of manure was very large, for instance around the nesting places of birds, there were only very few species, especially *Phormidium autumnale;* this was the case, too, near dunghills and liquid manure, where the same species and *Prasiola crispa* throve well; here too some species of Diatoms were added. Around the farmsteads, where manure is always found in greater or less amount, there appeared a vegetation rich in species of all algal groups, but especially Chlorophyceae and Diatoms, and among the latter a series of species which only occurred here and not on the natural, more purely mineral soil. The algal vegetation of the latter seemed rather to depend on the very variable conditions of moisture (BOYE PETERSEN 1928 II, p. 13), since, this apart, the soil in Iceland is of a rather uniform character.

The first rational experiments designed to establish the significance for the algae of the content of nourishment in the soil are due to GISTL (1933) and have been recorded above (pp. 16—17). Manured soil is much richer in species than the unmanured, and another result to be noted is that manure containing ammonium sulphate seems especially to favour the development of Diatoms.

It has repeatedly been observed in this country that after the ground has been strewn with "Nitrophoska", the surface is coloured green with algae after some time. Thus Dr. D. MÜLLER has brought me a sample of soil from Jutland in which I ascertained the presence of numerous round cells of green algae, and Dr. F. BØRGESEN has observed the same phenomenon in his garden at Hellebæk. In a soil sample which he brought me the following species occurred in great numbers:

Trichomes of Cyanophyceae (*Cylindrospermum* sp.?), *Navicula Atomus*, *Navicula gibbula* var. *terrestris*, *Nitzschia* sp., *Pinnularia obscura*, *Cylindrocystis Brebissonii*, *Hormidium flaccidum*, *Keratococcus bicaudatus*, *Vaucheria* sp. (*hamata*?).

It would seem quite obvious, then, that the soil's content of organic as well as inorganic nutrient substances is of great importance for the growth of the algae. In this field, however, we are only at the beginning of our investigations.

In connection with the question as to the importance of the nutrient substances in the soil for the algae, I shall touch upon another problem which has often been discussed and has given rise to a very comprehensive literature, viz. the problem whether algae are able to assimilate the free nitrogen of the air.

This literature up to 1914 has been carefully recorded by SCHRAMM (1914, p. 158 ff.), to whom I can refer the reader. The earlier authors used impure cultures in which bacteria were present, and the results differed. Some found a vigorous assimilation of nitrogen both in Chlorophyceae and Cyanophyceae, others found none. This circumstance may be explained in various ways. Partly it may be assumed that not all the algae investigated are capable of assimilating free N, but that this capacity is only found in a small number of species, similarly as in the bacteria, among which it is likewise quite few species that possess this capacity (SCHRAMM 1914, p. 157), partly it may also be supposed that N-assimilating bacteria happened to be present in some cultures, in others not, which may also explain the different results (KOSSOWITSCH 1894, p. 113). As SCHRAMM notes (1914, p. 172), the negative results of these earlier investigations with impure cultures are more reliable than the positive ones.

The first to use a pure culture of an alga for such investigations was KOSSOWITSCH (1894). He calls the alga *Cystococcus*, but from the description (l. c. p. 100) it is more probably a *Chlorella* sp., nearly allied to *C. vulgaris* Beyerinck. In a pure culture it was not able to assimilate N, whereas he finds, as usual, that cultures containing algae and bacteria can assimilate nitrogen from the air, especially when dextrose is present. KOSSOWITSCH (1894, p. 113) also explains the experiments of SCHLOESING et LAURENT (1891) with mixed cultures of various algae and bacteria, some of which showed N assimilation, others not, by the fact that the former have contained nitrogen fixing bacteria, the latter not. KOSSOWITSCH therefore thinks that there is a symbiotic relationship between the soil algae and the N-assimilating bacteria, the algae forming carbohydrates, which are utilised by the bacteria as a source of energy for an assimilation of nitrogen. Similar results to those of KOSSOWITSCH were obtained by several other authors with various green algae in pure cultures, especially SCHRAMM (l. c.), who found no N assimilation in 7 species.

WANN (1921), however, later took up the question for renewed examination in a series of (7) species of Chlorophyceae (principally from soil) in pure cultures free from bacteria and found, when there was nitric nitrogen and glucose in the cultures, an increase in the amount of nitrogen in them, and all the algae investigated behaved in the main identically. For *Protococcus* sp. (derived from bark) and *Protosiphon* it was doubtful, however. On the other hand, when ammonium sulphate or various organic substances were given as the nitrogen supply, no nitrogen fixing could be observed.

BRISTOL and PAGE (1923) have, however, criticised WANN's investigations and shown that his results were due to defective chemical methods. In a simple Kjeldahl determination some N is lost, when nitrate is present in the substance. In order to avoid this loss the substance may be subjected to an anterior treatment; but the method adopted by WANN did not exclude the loss of N when nitrate was present. In certain of the control flasks the nitrogen was found exclusively in the form of nitrate; here therefore the loss of N was especially great. In the corresponding culture flasks the greater part of the nitrate had been changed into organic nitrogenous compounds and hence the loss was considerably less. Accordingly it looked as if there had been an increase in these of the amount of nitrogen as compared with the control flasks. In the control flasks where the nitrogen was present as ammonium nitrate the loss of nitrogen during the analysis was less, and the difference between the N content of the control flasks and that of the cultures was therefore only half as large. With the employment of all the other sources of nitrogen no evidence of N assimilation appeared in his experiments.

BRISTOL and PAGE repeated WANN's experiments with a pure culture of a soil alga; with the adoption of a correct method of analysis it was not possible to detect any increase whatever in the nitrogen content of the cultures.

In all cases in which pure cultures without bacteria have been employed the Chlorophyceae have proved unable to assimilate free nitrogen. Since only a comparatively small number of species have so far been subjected to such investigations, it cannot of course be said with absolute certainty that nitrogen fixing green algae do not exist; but by degrees it has been shown that it is not very probable.

As far as the Cyanophyceae are concerned, there has been the difficulty that it has proved extremely troublesome to produce pure cultures of them free from bacteria. The earlier investigators who worked with cultures containing bacteria often found here a considerable assimilation of nitrogen (SCHLOESING et LAURENT 1892 I, II). Later BOUILHAC (1896) found that various blue-green algae (*Schizothrix lardacea*, *Nostoc punctiforme*) would not grow in cultures which he himself regarded as pure cultures when no nitrogenous compounds were present. If, on the other hand, he added some drops of a soil suspension, they began to grow vigorously. He infers that algae cannot assimilate free nitrogen, but that soil bacteria can do so by the aid of the algae.

GLADE, too, found (1914, p. 325) that species of *Cylindrospermum* cannot grow on a nitrogen-free substratum. HEINZE (1906 II, p. 897), on the other hand, alleges that *Nostoc* is able to fix free nitrogen. He was not able to obtain the alga in an absolutely pure culture, but only one organism caused an impurity, viz. a Streptothrix-like fungus, while *Azotobacter* were not present.

Streptothrix, however, he obtained in a pure culture, and it then turned out that this alone could not fix free N. *Nostoc* + *Streptothrix*, on the other hand, possessed this capacity, especially when phosphate was supplied in the form of K_2HPO_4 or K_3PO_4. *Nostoc*'s fixation of nitrogen was, however, far less than that of *Azotobacter*.

Of recent years various authors have alleged that they have obtained Cyanophyceae in pure culture and also that they have been able to show that they can fix free nitrogen. Thus MOLISCH (1926, p. 114) found that *Nostoc* from *Blasia* and *Caricularia* had a decided capacity for fixing nitrogen when cultivated in the light in a nitrogen-free mineral solution, and DREWES (1928) found the same for *Anabaena variabilis*, *Anabaena* sp., and a form nearly allied to *Nostoc punctiforme*.

ALLISON and MORRIS (1930 I and II) likewise realised *Anabaena variabilis* in a pure culture and found that it assimilated free nitrogen in the light in a nitrogen-free liquid medium through which was blown sterile air purified from ammonia. The addition of saccharose increased the fixation of nitrogen, and an increase of the amount of CO_2 in the air to 1 p.c. had the same effect. In the latter case the addition of saccharose caused no further increase.

On the other hand they obtained negative results with a series of Cyanophyceae from PRINGSHEIM, which, however, were not all in pure cultures free from bacteria. These were *Phormidium luridum* var. *olivacea*, var. *violacea*, *P. autumnale* var. *olivacea*, *P. favosum*, *P. laminosum* var. *aeruginea*, *Oscillatoria amoena*, *Lyngbya aerugineo-coerulea*, *Porphyridium cruentum*.

It would appear, then, that but few genera, especially *Anabaena* and *Nostoc*, are capable of fixing nitrogen, and they can only do so in the light, not in darkness. They cannot, therefore, be supposed to play any part in the fixation of nitrogen in the soil when they occur subterraneously.

In contrast herewith JESSIE JONES (1930) has found in the sheaths of nitrogen-fixing Cyanophyceae (*Nostoc*, *Rivularia*, *Gloeocapsa*) precisely such bacteria as are known to be able to utilise free N, viz. *Azotobacter chroococcum*, *Clostridium Pasteurianum*, and *Bacillus radicicola*. She therefore adopts the same view as those authors who suppose that there is a symbiotic relationship between the algae and the bacteria, and thinks that the Cyanophyceae themselves cannot assimilate nitrogen, but their symbiosis with the bacteria explains how they can grow on soil very poor in nourishment. The investigations of JESSIE JONES do not of course exclude the possibility that the algae can fix free nitrogen even without the cooperation of the bacteria.

O. RICHTER (1906, p. 58) found that *Nitzschia Palea* and *Navicula minuscula* cannot assimilate free nitrogen. This is, as far as I know, the only available information about the possibility of nitrogen fixation by Diatoms.

We thus arrive at the result that probably only a few genera of the Cyanophyceae are capable of fixing free nitrogen, and only in the light. On the other hand, it has been plainly shown that other algae, too, especially Chlorophyceae, may favour the development of nitrogen-fixing bacteria. To all appearance this process, too, will only take place in the light, from which it must be inferred that the subterranean algae are of no importance in this respect.

There is the fundamental difference between the nitrogen fixation of the blue-green algae and the bacteria that the former utilise solar energy (direct or indirect) which they absorb themselves, whereas the bacteria receive their energy supply from organic substances in the soil (cf. BEYERINCK 1901).

5. Survey of the Biology of the Soil Algae.

Soil algae occur on the surface of the soil (epiterraneously) as well as in the lower layers of the soil (subterraneously). I have found that this applies both to cultivated and uncultivated soil, and even to absolutely virgin soil in East Greenland.

Even though it has been proved of several soil algae that they can subsist mixotrophically in cultures (Cyanophyceae, Diatoms), or even heterotrophically (Chlorophyceae), nevertheless the available information would seem to indicate that under natural conditions they must in the main 'subsist autotrophically, since the subterranean algae do not multiply appreciably in the soil in darkness. For the Chlorophyceae that are able to live on organic substances in the dark this may be caused by the fact that precisely the substances which they need are not present in the soil, or that they are consumed so rapidly by bacteria that the algae cannot utilise them.

The predominant growth of soil algae occurs on the surface, in the light, and here, at any rate in the spring, a much larger number of algal germs are found than in the lower layers. It is highly probable that the algae multiply very rapidly precisely in the spring, but that the growth is arrested towards the middle of the summer owing to drought and strong insolation, and that many cells even die during this season; this may particularly be supposed to be the case with Diatoms and certain Cyanophyceae (Nostocaceae) and Chlorophyceae. It is possible that renewed intense multiplication takes place in the autumn, but for the present little is known about this. In dry periods most of the algae on the surface of the soil will undoubtedly pass into more or less marked resting stages. Filamentous algae such as *Hormidium* and *Bumilleria* will be dissociated into individual cells with the alternation of drought and moisture.

A period of humidity after drought will in many Chlorophyceae start a formation of zoospores or gametes. These swarming cells will move away from the place where they have been formed; but in most cases they will rather soon come to rest again in the light since in most Chlorophyceae they are negatively photokinetic. Still in some instances they will perhaps as a result of positive photokinesis work their way into the soil and come to rest there.

Drought will presumably be able to kill off a considerable percentage of algal cells in the surface, especially if it sets in rapidly; but there can be no doubt that there will always be a larger or smaller number of them which owing to changes in the protoplasm will be able to resist the influence of desiccation. The algal germs occurring in dry soil samples will keep alive and be able to germinate for years. A number of algae, e. g. *Vaucheria* and Nostocaceae will be killed off entirely during long periods of drought, with the exception of resting spores.

Cold will perhaps be able to kill some algal cells; but it will hardly ever be so severe, at any rate in Denmark, that it will be able to kill off all algae on the surface.

Strong sunlight in connection with the high temperature which it may produce in the surface of the soil will be tolerated by most soil algae. Diatoms and perhaps certain Cyanophyceae may perhaps use their motility for reaching a safe place some little way below the surface (negative phototaxis towards high intensities of light).

On the other hand, both the data from literature on the tactic reactions of freely moving algae and my own direct experiments would seem to show that algae do not move actively into the deeper layers of the soil.

By rain water, perhaps also by the aid of earthworms, and on cultivated soil when it is turned over, algae are constantly carried down into the earth; they have been shown to be present right down to a depth of 2 m; but there is a rapid decrease in numbers as we pass downwards. It must be supposed that it is especially heavy showers coming on suddenly after droughts which are able to carry down the algae, and that the process, therefore, does not take place steadily but on the contrary very intensively in some few brief periods, and perhaps not at all during long periods.

Algae will survive very long within soil in the dark (80 years); this sufficiently explains the fact that algae are always present in the lower layers of the soil, even if it may be assumed that the number carried down annually is not very large. Further, it would seem to have been proved that the algae within the soil occur for the most part in a vegetative state and not in the form of special resting stages.

In the main the subterranean algae must be supposed to be in a state of inactivity; but the possibility cannot be entirely dismissed that some of them may be able to multiply to a slight extent by absorbing organic substances from the soil. However, this multiplication will as a rule not be more rapid than that the number of new cells formed will equal the number of older ones dying off in the same period, and this keeps the number of living cells fairly constant.

The subterranean algae will always have a sufficient supply of moisture, and fluctuations of the temperature will be more or less toned down for them. Their life conditions will therefore be more uniform, without extremes, 'and this may be conceived to cause a low mortality among them. According to the available information the numbers of surface algae vary much with the seasons, whereas the number of algae within the soil is more constant.

On cultivation of the soil the algae from the deeper layers will again be able to come up to the surface. But even in uncultivated soil it is possible that a small number of germs may come up to the light again, for instance by the activity of earthworms;

a few observations would seem to show that algae can pass through the intestine of an earthworm without perishing.

Nor can the possibility be dismissed that algae from the deeper layers may wander actively up to the surface, since a number of algae have shown negative geotaxis. ESMARCH's experiments have proved that algae from the upper layers of the soil will penetrate through a piece of filter paper covering the surface. Conversely, many algae capable of motion have proved negatively phototactic with high illumination. There is, then, reason to believe that in the top millimetres of the soil the algae capable of motion will be able to wander up or down according to the changes of the climate.

VI. The Relation of the Soil Algae to Chemical Processes in the Soil.

Investigators of the soil algae of course early formed opinions as to their importance for the chemical processes in the soil. The opinions of a given period are marked by the knowledge of that period, hence they have since undergone considerable change. It was especially when the existence of subterranean algae was discovered that inquirers began to speculate as to what might be their significance for the soil (RUSSELL 1923, p. 114), and it became quite common to ascribe no small role to them in the metabolism of the soil, even though one had nothing but conjectures to go by, which were not supported by conclusive experimental results. I shall not here attempt to describe the development of the various views, it will suffice to show what idea of the function of the algae one must form from the earlier and later data presented in this work. A general view of what is at present commonly supposed about this question may be obtained through WAKSMAN (1931, p. 218) and in more detail in BRISTOL ROACH (1927 III, 1928 II, p. 344).

FEHÉR (1933, p. 174) has attempted to calculate the total weight of the algae occurring in the soil as compared with the total weight of bacteria in the same amount of soil. As a general norm he supposes that the soil contains 50,000 algal cells, globular, 5 μ in diameter, and 10,000,000 bacteria, globular, 1 μ in diameter, per gr. of soil. If the specific gravity of the living cells is equalised with that of water, he arrives at the following result:

Weight of algae	3.27 mgr per gr of soil
Weight of bacteria	5.23 — — —

Hence the amount of algal matter in the soil is by no means slight. It is another question in how far this algal matter is able to start chemical processes in the soil. In that respect the algae will no doubt fall far short of the bacteria, amongst other things on account of the smaller size of the bacteria, and thus greater surface compared with the mass. I shall refrain from making any calculation, but no one can doubt that with the figures assumed for algae and bacteria by FEHÉR, the effect of the former on the chemical processes in the soil will be slight in comparison with that of the latter.

However, there is evidence available to show that the number of soil organisms, bacteria as well as algae, in favourable cases may be much larger than assumed by FEHÉR. Thus KOFFMANN (1934, p. 278) arrives at much higher figures by his direct method of investigation. In 1 gr of soil he finds of bacteria a total of 1.3 milliards having roughly a total weight of 5 mgr. The number of algae is considerably less. In 1 gr of soil he finds 290,000 "cell fragments" (I am not quite sure of what he means by this) and about 1 million "algal cysts". Thus even if the total weight of the bacteria according to KOFFMANN is about the same as that calculated by FEHÉR, their physiological effect will undoubtedly be much more considerable than could be computed from FEHÉR's figures, when the 5 gr of bacteria consist of 1.3 milliards of individuals as against FEHÉR's 10 millions. Even if KOFFMANN's "cell fragments" and "algal cysts" are added, the proportionate number of algae and bacteria will according to him be as $^1/_{1000}$, whereas the corresponding ratio according to FEHÉR will be $^1/_{500}$. This would seem to show that the algae are of less importance in the economy of the soil than might be expected from FEHÉR's computation.

In the present work I have found up to 3,000,000 algae per c.c. of soil in the surface of cultivated soil. This number was found in a place where there was no macroscopically visible algal vegetation on the ground. Where such a vegetation is present, the number of algae must be assumed to be much larger, and it is then possible that the algae may exert an influence besides that of the bacteria on the chemical processes in the top layer of the soil.

FRANCÉ states (1913, p. 93) that German farmers consider "grüne Schimmel auf der Erde" a sure sign of abundant crops; thus they seem to have learned in a purely practical way that a copious algal vegetation on the ground has a favourable influence on the amount of the crops. Even though no weight can be attached to this as a scientific observation, it is worth noticing, for this kind of experience from old times has often proved to contain a nucleus of truth.

There is one more factor I would mention in this connection. Much would seem to show that there are great seasonal variations in the numbers of algae within the soil (BRISTOL ROACH 1927 I, p. 572, FEHÉR 1933, p. 176). This applies chiefly to the surface algae which multiply rapidly under favourable conditions, while, on the other hand, many perish in unfavourable periods (frost, drought). This will undoubtedly involve a vigorous chemical activity.

It is quite otherwise with the subterranean algae, the number of which seems more constant all the year round and altogether comparatively low. Below a depth of 5 cm no one has as yet found higher figures than 55,747 germs per gr of soil (BRISTOL ROACH 1927 I, p. 572), while in my Danish samples I have not found more than 20,000 germs per c.c. of soil. As a consequence it must be supposed that the influence on the chemical processes in the soil of these algae will be slight, and below a depth of 20 cm they generally occur in such small numbers that they can be quite disregarded as a factor of importance (see above p. 49 and BRISTOL ROACH l. c.).

This author has stated clearly that a distinction must be made between the epiterranean and the subterranean algae, since they will in the main have contrary effects on the soil. On the surface the algae act as producers, within the soil as consumers.

The organic substances which the epiterranean algae form by assimilating carbon dioxide will be able to enter in various ways into the chemical cycles of the soil. FRANCÉ (1913, p. 86) has observed that soil rhizopods live chiefly on soil algae, and he further thinks that the latter form no inconsiderable proportion of the food of earthworms which eat the algae with the soil. How far this is correct I am unable to decide. But so much is certain that sooner or later the organic substances by the death of the algae will be able to serve as nourishment for other organisms and in that way possibly be changed into substances useful to higher plants.

In a report from Rothamsted Experimental Station for 1923—24 (see Journ. of Bot. **63**: 366) it is pointed out that as far as the mineral foods of the soil are concerned the algae compete with the higher plants. In the first instance they will thus deprive these of some food, but only from the surface in any appreciable amount. The amount of mineral food which algae may be supposed to absorb from the layers of the soil where the higher plants have their roots will no doubt be minimal since no noticeable growth of the algae occurs here.

On the other hand it is possible that the surface algae by assimilating a number of nutrient salts may contribute to retain these while otherwise they would perhaps be leached from the soil (BRIERLEY 1928, p. 11). In this connection I may remind the reader that GAUTIER et DROUIN (1888, p. 1099) found that algae within the soil change ammonia to organic nitrogenous compounds; they thought therefore that they are of importance by their absorption of gaseous ammonia which is continually given off from the soil and thus prevent a loss of nitrogen from it. Sooner or later the soil must again profit by the substances, fully or in part, when the algae die and their substances are desorganised again.

A point which has proved of special interest is the relation of the algae to the nitrogen cycle of the soil, and an extensive literature is available on this subject, from which we shall attempt to select some main points. As shown at more length above, it is probably only some few genera of Cyanophyceae that are capable of assimilating free nitrogen of themselves, whereas long series of investigations show that soil containing algae and bacteria is able to fix considerable amounts of nitrogen in the light (FRANK 1888, 1889; SCHLOESING et LAURENT 1891, 1892 I and II; KOCH und KOSSOWITSCH 1893; L. RICHTER 1899; DÉHERAIN et DEMOUSSY 1900; BOUILHAC et GIUSTINIANI 1903, 1904; HEINZE 1906 II; WILFARTH und WIMMER 1907).

In the dark, on the other hand, no algae will develop, and no fixation of nitrogen will occur. (FRANK 1889; KOCH und KOSSOWITSCH 1893; L. RICHTER 1899; cf. also ALLISON and MORRIS 1930). It follows that only the surface algae bear any relation to the nitrogen fixation, whereas the subterranean algae, in relation to the nitrogen compounds, too, only appear as consumers.

It is generally supposed that the importance of the algae to the nitrogen fixation of the bacteria is due to the fact that as producers they procure carbohydrates or other nutrient substances which may serve as a source of energy for the bacteria. BRISTOL ROACH, however, throws out the suggestion (1927 III, p. 8) that their stimulating effect on the nitrogen fixation of the bacteria may be due to the fact that the algae quickly use up the nitrogen compounds produced by the bacteria, since these when accumulated in the earth have been shown to reduce the activity of the bacteria. Hence it may be that the algae here act as a necessary intermediate link, rendering possible an accumulation of organic nitrogen compounds in the soil.

Already FRANK (1888, p. 551) thought that the algae had a brief lifetime in the soil, being quickly replaced by new ones, so that in his opinion they came to act as a constant source of manure.

BOUILHAC et GIUSTINIANI (1904) held similar views, and they even thought that the inoculation of the soil in their experimental vessels with algae and bacteria had the same effect on the growth of higher plants as a good dose of sodium nitrate. They found also that the amount of nitrogen at the surface and at the bottom of the culture vessels was about the same at the end of the experiment, from which they inferred that the nitrogen compounds formed were rapidly diffused in the soil, so that the higher plants could derive benefit from them.

On the other hand, the experience of DÉHÉRAIN et DEMOUSSY (1900) as also of WILFARTH und WIMMER (1907) is quite different. They found that the nitrogen in their cultures was only present in the algal layers, whereas the portion of sand free from algae in the culture vessels contained only minimal amounts of nitrogen.

FRANK has derived fresh support from ALLISON and MORRIS (1930 II), who have arrived at the strange result that the clear, filtered liquid from their cultures of *Nostoc* and *Anabaena* in a liquid medium containing organic salts without nitrogen, after the experiments contained no slight amount of nitrogen compounds in solution. Whether these originate from dead cells whose matter has commenced to be disintegrated, or whether the living algae are actually able to secrete nitrogenous substances, they leave open to conjecture.

What are the actual facts of the matter can hardly now be decided; but it must be taken for granted that the substances accumulated in the algal cells sooner or later must pass into compounds in which they can be profitable to the soil and the higher plants growing in it.

Summing up, then, we arrive at the result that the epiterranean algae will in the main be useful in the soil by 1) producing organic matter, and 2) either by assimilating free nitrogen themselves or by stimulating the nitrogen fixing activity of the bacteria and thus contributing to increase the nitrogen content of the soil.

Investigators have early ascribed importance to the soil algae as preparers of new ground for higher plants. We have mentioned this above at pp. 8—9, to which we refer the reader. I shall merely add that BEYERINCK (1901) considers blue-green algae as the only known organisms which are able to synthetise their organic substances from carbon dioxide and free N, which makes them especially adapted to prepare new soil, as described by TREUB (1888), GRAEBNER (1895) and others.

FEHÉR (1933, p. 167) sees in the algae's assimilation their chief biological function in the forest ground; he thinks that in this way they contribute oxygen to the heterotrophic organisms of the soil. This view, however, is probably connected with FEHÉR's conjecture that the subterranean algae, too, have a photosynthetic carbon dioxide assimilation, which, as mentioned above, must presumably be regarded as excluded.

On the other hand, HARRISON and SUBRAMANIAN AYIER's allegation (1913, 1914) that algae in the rice fields are very useful in forming oxygen, which may serve for the respiration of the roots of the rice, is more probably correct. When the soil is undrained, the oxygen produced cannot penetrate very far into it, and the roots of the rice must therefore spread very superficially; if, on the other hand, the earth is drained, the water containing oxygen will penetrate far deeper into the soil, the roots of the rice will follow, and thus be able to derive more advantage from the soil.

The subterranean algae must be regarded exclusively as consumers of inorganic as well as organic matter; but they are probably of slight importance, partly because they do not occur in large numbers, partly because most of them are in a state of inactivity in the soil, during which their vital processes are reduced to a minimum.

bye's specimen in the Botanical Museum the presumed algae turned out to be hairs on the rhizome.

The same species are included by Hornemann in the second edition of Dansk oeconomisk Plantelære (1837); but no new ones are added. Hornemann's attempt to give these algae Danish names, e. g. omskedet Host Bævrenaal, = *Phormidium autumnale*, seems droll to the modern reader.

Two small works by Liebmann from his young days must be regarded as a final result of this period of brilliant activity for algology in Denmark. In his paper of 1839 he adds a couple of species to the soil algae already known, viz. *Scytonema Myochrous* and *S. variegatum*, which, however, are probably both = *Stigonema ocellatum*, and *Scytonema Bangii*, which is = *Schizothrix Friesii*. Several of the soil algae mentioned are figured in Flora danica, especially in the period 1775—82, when O. F. Müller was the editor, and in the period 1806—1840, when Hornemann edited it. In all, there are figures of 18 algal species, which are presumably soil algae, as also of *Conferva velutina* (= a moss protonema) and *Mycinema Pteridis* (= hairs on the rhizome of *Pteridium*).

Both O. Fr. Müller and Lyngbye mention Diatoms, but none from the surface of the soil. Only Heiberg, who published Conspectus Diatomacearum danicarum in 1863, says in his introduction (p. 22) that "on damp soil, on the sides and surfaces of damp flowerpots and in similar places many smaller species of Diatoms are met with"; but he does not mention one of these species.

J. P. Jacobsen, who wrote about Denmark's Desmidiaceae (1875—76), says about *Penium Brebissonii* (*Cylindrocystis B.*) that it occurs in "localités humides". Johs. Schmidt issued in 1899 a collective list of the Cyanophyceae of the group Hormogoneae found in Denmark. Without going more thoroughly into their biology Schmidt yet distinguishes between marine, freshwater, and land forms, and he gives in all about 25 species of Hormogoneae as soil algae.

At the beginning of the 20th century Østrup worked for a number of years on the Danish Diatoms, and in 1910 he published a complete list of what he had found. Østrup did in the main purely systematic work, still several Diatoms from the surface of the soil are given in his work. I have previously (1915, p. 297) brought together these species in a separate list, so I shall here say no more on this subject.

The first work in which the Danish soil alga flora has been treated from an ecological point of view is BOYE PETERSEN (1915). Since that work as well as others by the present writer are cited elsewhere in this treatise, I shall here merely mention that BOYE PETERSEN (1932 I, II), too, has treated Danish soil algae.

b. List of Soil Algae met with in Denmark and in East Greenland.

According to the original plan the succeeding list of Danish soil algae was intended to comprise the known *euterrestrial* species. I have, however, included a number of soil algae which occur more occasionally in company with the latter, even though their main distribution is in other algal communities. Against each species it is stated to which biological group or groups among those mentioned in the introduction it must be supposed to belong. For each species is cited as far as possible all works in which it is mentioned in the Danish botanical literature, as well as all places in the foreign literature in which it is expressly stated to have been found on or in soil, with the exception only of floristic works making more vague statements ("on soil", "on damp soil", and the like), and the earlier literature, in which it is very difficult to see from the names given what species are meant.

As mentioned above, JOHS. SCHMIDT (1899) has, as far as the Cyanophyceae are concerned, given the habitats of the individual species in general, while for the Danish localities mentioned there is as a rule no information about the nature of the habitat. In my list all the species have been included about which SCHMIDT states that they may occur on soil, even though it does not appear clearly that they have been found on this substratum in Denmark.

Finally my list also includes the species I found in cultures of soil samples from East Greenland.

CYANOPHYCEAE

I. Coccogoneae.

I. Synechococcus.

Synechococcus aeruginosus Näg. Nägeli 1849, p. 56, tab. 1 E, fig. 1; Esmarch 1911, p. 71; Boye Petersen 1928 II, p. 11; 1932 I, p. 14.

Observed in cultures of soil samples from East Africa, Iceland, and Denmark (Hammer Bakker). The species is mentioned as occurring on moist rocks, in Sphagnum moors, on snow, and in therms? (Geitler 1930—32, p. 274).

Is then presumably pseudoaërial — hydroterrestrial.

II. Hormogoneae.

1. OSCILLATORIACEAE

I. Oscillatoria.

Oscillatoria anguina Bory; Gomont Monogr. II, p. 214, pl. VI, fig. 16; Esmarch 1914, p. 265; Boye Petersen 1923, p. 274.

As far as is known, the species occurs in the main in fresh water, stagnant as well as running. It is found in cultures of soil samples from Germany and East Greenland.

Hydrophytic — hydroterrestrial.

— **janthiphora** (Fior.-Mazz.) Gomont; Gomont Monogr. II, p. 224, pl. VII, fig. 20, 21.

In a culture of a soil sample from East Greenland. The species originally found on Hydrurus in brooks.

Is presumably hydrophytic — pseudoaërial.

— **splendida** Grev. Gomont Monogr. II, p. 224, pl. VII, fig. 7, 8; Moore and Karrer 1919, p. 304; Boye Petersen 1928 II, p. 11, 17.

Known from cultures of soil samples from East Greenland, North-America, Iceland. The species otherwise known to grow on mud and water plants in stagnant water and on hot house walls. It must probably be classed as hydrophytic — pseudoaërial.

— **sancta** Kütz. Gomont Monogr. II, p. 209, pl. VI, fig. 12; Schmidt 1899, p. 352; Boye Petersen 1923, p. 273.

Never found in cultures of soil samples. The species is most frequently found in fresh water, but it also occurs on earth in hot-houses. Thus it is known from the Botanical Garden, Copenhagen. Hydrophytic — hydroterrestrial.

— **sp.** Indeterminable Oscillatoria-trichomes are found in cultures of soil samples from Amager (garden soil) and East Greenland.

II. Phormidium.

Phormidium autumnale (Ag.) Johs. Schmidt emend. Gomont Monogr. II, p. 187, pl. V, fig. 23, 24; Schmidt 1899, p. 348; Esmarch 1914, p. 265; Bristol 1920, p. 59; 1927 I, p. 569, 576; Boye Petersen 1923, p. 283; 1928 II, p. 11, 17; 1932 I, p. 15; Fehér 1933, p. 168; *P. uncinatum* Gomont Monogr. II, p. 184, pl. V, fig. 21, 22; Esmarch 1911, p. 72; Singh 1933, p. 106; *Oscillatoria autumnalis* Lyngbye 1819, p. 95; Hornemann 1837, p. 648.

Is one of the commonest algae on and in the soil. Often it forms macroscopically visible growths on the earth, in particular where this is strongly manured. Further it is found in fresh water, stagnant and running.

Recorded from cultures of soil samples from East and S.-West Africa (*uncinatum*), Germany, England, Hungary, Iceland, East Greenland, India.

Euterrestrial — hydrophytic.

— **foveolarum** (Mont.) Gom. Gomont Monogr. II, p. 164, pl. 4, fig. 16; Esmarch 1911, p. 72; Boye Petersen 1932 I, p. 15; Singh 1933, p. 106; Fehér 1933, p. 168.

At times the species forms vegetations on the soil and on limestone rocks. Further it is found in water.

Recorded in cultures of soil samples from East and S.W. Africa, Finland, East Greenland, India.

From Denmark: Hammer Bakker, Ermelunden, Botanical Garden.

Probably mainly euterrestrial — pseudoaërial.

Phormidium tenue (Menegh.) Gom. Gomont Monogr. II, p. 169, pl. IV, fig. 23—25; Esmarch 1914, p. 266; Bristol 1919 II, p. 106; 1920, p. 59; Moore and Carter 1926, p. 117; Bristol Roach 1927 I, p. 569.

To this species are referred different forms, found under very varying conditions: therms, freshwater, brackish water, soil. As the investigation of this species is very difficult because of its extremely slender trichomes, it may be doubted whether all these forms are justly referred to the same species. A critical comparison of them is certainly called for.

The species is found in cultures of soil samples from: Germany, England, N. America (1 foot depth), East Greenland.

Euterrestrial — hydrophytic?

— Valderiae (Delp.) Schmidle; Geitler 1925, p. 381, fig. 481; *P. Valderianum* Gomont Monogr. II, p. 167, pl. IV, fig. 20.

The species is known as growing in water of varying kinds. It is found in a culture of a soil sample from East Greenland (20 cm depth). Hydrophytic — hydroterrestrial.

III. Symploca.

Symploca muscorum (Ag.) Gom. Gomont 1890, p. 354; Monogr. II, p. 110, pl. II, fig. 9; Schmidt 1899, p. 337; Boye Petersen 1923, p. 286.

The species is known from all parts of the world. As a rule it grows among mosses on "moist" earth, but sometimes also in fresh water.

In Denmark known from different localities (Sealand, Jutland). Appeared in a culture of a soil sample from East Greenland (10 cm depth).

Hydroterrestrial?

IV. Plectonema.

Plectonema Battersii Gom. Gomont 1899, p. 36; Bristol 1919 II, p. 105. 1920, p. 65; Boye Petersen 1932 I, p. 15, fig. 1.

As *Plectonema Battersii* is a marine or submarine species, I have on a previous occasion (l. c.) expressed my doubt as to whether the soil alga found by Bristol (l. c.) and myself really is identical with this species.

The drawing of further conclusions on the basis of the occurrence of this marine species in the soil should, therefore, be deferred for the present.

The species exhibits great power of resistance against desiccation:

I have kept it in a dry state for 24 months, and Bristol (1919 l. c.) has stated that is was living in stored soil 50 years old, which during that time had been kept in darkness.

Found in cultures of soil samples from England and Denmark (Hammer Bakker, Botanical Garden).

Euterrestrial — hydrophytic?

V. Schizothrix.

Schizothrix Friesii (Ag.) Gom. Gomont Monogr. I, p. 316, pl. IX, fig. 1, 2; Schmidt 1899, p. 328, fig. 4; Boye Petersen 1923, p. 290; *Scytonema Bangii* Lyngbye 1819, p. 98, tab. 28; Fl. dan. 1818, tab. 1602, 1; Liebman 1839, p. 489.

Never seen in cultures of soil samples. On the other hand it is most frequently found forming macroscopically visible growths on the soil. It is questionable whether it can also occur in water.

Known from Denmark from several localities in Sealand and Jutland.

Euterrestrial.

— **fuscescens** Kütz. Kützing 1843, p. 230; Gomont Monogr. I, p. 324, pl. XI, fig. 4—6; Schmidt 1899, p. 329.

Met with only once in Denmark (Jutland).

Euterrestrial?

— **lateritia** (Kütz.) Gom. Gomont Monogr. I, p. 308, pl. VIII, fig. 4, 5; Schmidt 1899, p. 327, fig. 3.

Hardly ever found on the soil in Denmark.

Pseudoaërial — hydrophytic?

— **purpurascens** (Kütz.) Gom. Gomont Monogr. I, p. 320, pl. IX, fig. 6—8; Schmidt 1899, p. 329.

Found only once in Denmark (Bornholm).

Euterrestrial?

VI. Microcoleus.

Microcoleus vaginatus (Vauch.) Gom. Gomont 1890, p. 353; Monogr. I, p. 355, pl. XIV, fig. 12; Schmidt 1899, p. 333; Esmarch 1914, p. 266; *Oscillatoria vaginata* Vaucher 1803, p. 200, pl. 15, fig. 13.

The species known from most parts of the globe. It is reported as growing on "moist" earth, but also submerged in fresh water.

Has developed in a culture of a soil sample, which had been preserved in a dry state for 36 months.

In soil cultures from Denmark: Botanical Garden, Holmsland (pasture).

Euterrestrial — hydrophytic.

2. NOSTOCACEAE

I. Nostoc.

Nostoc commune Vaucher: Vaucher 1803, p. 222, tab. 16, fig. 1; Bornet et Flahault Revision IV, p. 203; O. Fr. Müller 1767, p. 216 (*Tremella Nostoc*): Fl. dan. 1782, tab. 885, 1 (*Tremella Nostoc*); Hornemann 1796,

p. 694 (*Tremella Nostoc,* rodløs Levresvamp); Lyngbye 1819, p. 198, tab. 68; Hornemann 1837, p. 627 (almindelig Troldsmør); Schmidt 1899, p. 363; Esmarch 1911, p. 73; Bristol 1920, p. 60; Boye Petersen 1923, p. 294; Moore and Carter 1926, p. 117; Fehér 1933, p. 168.

This species is one of the oldest known soil algae, and is probably spread all over the globe. For Denmark it is known from all parts of the country. It frequently grows directly on the soil, and also on "dripping rocks", but only exceptionally in water.

Recorded from cultures of soil samples from: East Africa, N. America (down to 1 foot 5 inches' depth), England, Finland, Norway.

Euterrestrial — pseudoaërial.

Nostoc ellipsosporum (Desmaz.) Rabh. Bornet et Flahault Revision IV, p. 198; Schmidt 1899, p. 363; Esmarch 1911, p. 72; 1914, p. 266; Singh 1933, p. 106.

Not found in cultures of soil samples in Denmark. But it occurred in cultures of soil from East Africa, India, and Germany. Apparently it occurs only in soils with a great amount of moisture.

Pseudoaërial — hydroterrestrial.

— **punctiforme** (Kütz.) Hariot; Hariot 1891, p. 29; Schmidt 1899, p. 361; Esmarch 1911, p. 72; Francé 1913, p. 15; Esmarch 1914, p. 267.

The species is mentioned as growing in freshwater as well as in the soil and as an endophyte in certain higher plants. It is not established with certainty whether the endophytic and the freely living plants are identical (Geitler 1930—32, p. 835). Found in cultures of soil samples from East Africa, Samoa, Germany (down to 30 cm depth). Apparently it occurs only in soils with a great amount of moisture.

Hydroterrestrial — hydrophytic?

— **sphaericum** Vauch. Vaucher 1803, p. 223; Bornet et Flahault Revision IV, p. 208; Schmidt 1899, p. 364; Esmarch 1911, p. 73; Francé 1913, p. 15; Esmarch 1914, p. 267; Boye Petersen 1923, p. 294; Fehér 1933, p. 168; Singh 1933, p. 106; *Conferva Pisum* Fl. dan., tab. 660, 2.

The species occurs in freshwater as well as on land, most frequently on "dripping rocks". It has been found in cultures of soil samples from East Africa, S.W. Africa, India, Germany, Sweden. In the cases in which more precise data have been given, the samples were taken in very moist places.

Hydroterrestrial — pseudoaërial — hydrophytic.

— **sp.** In cultures of soil samples from Holmslands Klit (arable soil, 5—10 cm depth), Botanical Garden, and East Greenland (surface and 40 cm depth) sterile thalli of Nostoc are met with.

II. Anabaena.

Anabaena oscillarioides Bory; Bornet et Flahault Revision IV, p. 233; Esmarch 1911, p. 74; 1914, p. 270; Bristol 1919 II, p. 102; 1920, p. 63; Boye Petersen 1923, p. 300; Bristol Roach 1927 I, p. 569.

Not observed as a soil alga in Denmark; elsewhere apparently common in soil. Recorded in cultures of soil samples from East Greenland, East Africa, S.W. Africa, Germany, and England. The form found in this latter country is by Bristol (1919 II) named var. *terrestris*. It germinated after 59 years' rest and occurred in the soil down to 6 inches' depth.

Hydrophytic — euterrestrial?

Anabaena variabilis Kütz. Kützing 1843, p. 210; Bornet et Flahault Revision IV, p. 226; Schmidt 1899, p. 367; Esmarch 1911, p. 74; 1914, p. 270; Bristol 1920, p. 62; Boye Petersen 1923, p. 296; 1932 I, p. 14; Gistl 1933, p. 354, 367; Singh 1933, p. 106; Fehér 1933, p. 168.

This species is commonly looked upon as a brackish- and freshwater species. Nevertheless it has turned out to be very common in soil (down to 30 cm depth). It is found in soil-alga cultures from East Greenland, East Africa, S.W. Africa, India, Kiautschau, Samoa, Germany, England, Hungary, and Finland.

Within Denmark only met with as a soil alga in Hammer Bakker.

Hydrophytic — euterrestrial.

— **sp.** Indeterminable sterile filaments of *Anabaena* developed in cultures of soil from Holmsland (pasture, 5—20 cm depth), and the Botanical Garden; further in soil samples from East Greenland (0—30 cm depth).

III. Nodularia.

Nodularia Harveyana (Thwait.) Thuret. Bornet et Flahault Revision IV, p. 243; Schmidt 1899, p. 373; Esmarch 1911, p. 73; 1914, p. 269; Bristol 1919 II, p. 104; 1920, p. 62; Boye Petersen 1923, p. 301.

So far not found on soil in Denmark. According to the data in the literature the species possesses a very unusual power of adaptation to different conditions. It is met with in salt water, brackish water, fresh water, on soil and rocks, and even on the bark of trees.

Found in cultures of soil samples from: S.W. Africa, Germany (down to 20—25 cm depth), England.

Hydrophytic — euterrestrial?

IV. Cylindrospermum.

Cylindrospermum catenatum Ralfs. Bornet et Flahault Revision IV, p. 254; Schmidt 1899, p. 373; Esmarch 1911, p. 74; 1914, p. 270; Bristol Roach 1927 I, p. 569; Gistl 1933, p. 354, 367.

The species only once met with in Denmark (Aldershvile). As a soil alga known from East Africa, Germany, England. It is also found in the deeper layers of the soil (Esmarch 1914 down to 25 cm depth, Bristol 1927 I, down to 12 inches' depth).

Presumably hydroterrestrial.

— **licheniforme** (Bory) Kützing. Bornet et Flahault Revision IV, p. 253; Schmidt 1899, p. 372; Esmarch 1914, p. 270; Bristol 1919 II, p. 105; 1920, p. 63; 1927 I, p. 569.

Common as a cover on the soil; in this form known from Denmark (Sealand, Falster). Has been found in cultures of soil samples from

Germany (clayey and marshy soil down to 25 cm depth), and England. Developed in cultures of soil samples 60 years old (Bristol 1919 II).

Euterrestrial.

Cylindrospermum majus Kützing. Kützing 1843, p. 212; Schmidt 1899, p. 372, fig. 24; Esmarch 1911, p. 74; 1914, p. 270; Bristol 1920, p. 63; Singh 1933, p. 106.

Esmarch (1914) found this species in soil of very different kinds, and down to 40 cm depth (Germany). Further the species is known as a soil alga from East Africa, India, and England. Likewise it is probably of common occurrence in Danish soil.

Euterrestrial — hydroterrestrial.

— **stagnale** (Kütz.) Bornet et Flahault. Bornet et Flahault Revision IV, p. 250; Schmidt 1899, p. 371.

Presumably this species mainly occurs in fresh water, and only occasionally on moist soil. Never found in cultures of soil samples.

Hydrophytic — hydroterrestrial.

— **sp.** Sterile and thence indeterminable trichomes of *Cylindrospermum* species developed in cultures of soil from Holmslands Klit (dune depression), Holmsland (pasture, 10 cm depth), Botanical Garden. East Greenland.

3. SCYTONEMACEAE

I. Scytonema.

Scytonema Hofmanni Ag. Bornet et Flahault Revision III, p. 97; Schmidt 1899, p. 381; Esmarch 1914, p. 272; Moore and Karrer 1919, p. 304.

Apparently never met with on the soil in Denmark. Developed in cultures of soil samples from Germany, N. America (20 cm depth).

Pseudoaërial — hydroterrestrial?

— **ocellatum** Lyngbye. Lyngbye 1819, p. 97, tab. 28 A; Bornet et Flahault Revision III, p. 95; Boye Petersen 1932, p. 15.

Only once found in Denmark (Hammer Bakker).

Hydroterrestrial — pseudoaërial.

4. SIROSIPHONACEAE

I. Stigonema.

Stigonema minutum (Ag.) Hass. Bornet et Flahault Revision III, p. 72; Schmidt 1899, p. 409; Boye Petersen 1923, p. 312; 1928 II, p. 11; 1932 I, p. 14.

The species is most frequently found growing on dripping rocks, and among mosses on moist ground. Known from all larger parts of the globe. Within Denmark met with on Bornholm and in Hammer Bakker.

Pseudoaërial — hydroterrestrial.

— **ocellatum** (Dillw.) Thuret. Bornet et Flahault Revision III, p. 69; Schmidt 1899, p. 408; *Scytonema myochrous* Fl. dan. 1818, tab. 1602, 2; *S. variegatum* Liebman 1839, p. 488; Fl. dan. 1840, tab. 2315.

Thrives mainly in water, though often in bogs among Sphagnum. In Denmark found in Lyngby Mose.

Hydrophytic — hydroterrestrial.

5. RIVULARIACEAE

I. Calothrix.

Calothrix parietina (Näg.) Thur. Bornet et Flahault Revision I, p. 366; Schmidt 1899, p. 394; Esmarch 1914, p. 272; Boye Petersen 1923, p. 315.

It is doubtful whether this species is found on the soil in Denmark; on the other hand Esmarch (l. c.) has recorded it from Germany in cultures of a sandy heath-soil and a marshy soil. The species grows mainly on dripping rocks, and hence must be regarded as pseudoaërial.

DIATOMEAE

I. Centrales.

I. Melosira.

Melosira Dickiei (Thw.) Kütz. Van Heurck Traité, p. 444, pl. 19, fig. 623; Boye Petersen 1915, p. 296; Beger 1927, p. 390, 401; 1928, p. 383.

This on the whole rare species is only known from moss carpets, and is by Beger (l. c.) referred to his mesophilous type. In Denmark only once found on rocks with moss (Bornholm).

Pseudoaërial.

II. Pennales.

1. EUNOTIACEAE

I. Eunotia.

Eunotia gracilis (Ehr.) Rabh. Hustedt 1930, p. 185, fig. 253; Østrup 1910; p. 173; Boye Petersen 1915, p. 295; 1928 II, p. 14; 1928 I, p. 375, 1932 I, p. 16; Fehér 1933, p. 168; Beger 1927, p. 401.

Found in Denmark on rocks among mosses (Bornholm), in Hammer Bakker among *Sphagnum* in the spring moor, and as a full hydrophyte. This is also in accordance with its occurrence in Iceland, mainly in mýri and flói, and with the fact that it is referred by Beger to the mesophilous type.

Pseudoaërial — hydroterrestrial?

2. ACHNANTHACEAE

I. Achnanthes.

Achnanthes coarctata Bréb. Cleve Syn. II, p. 192; Van Heurck Syn. pl. 26, fig. 17—20; Østrup 1910, p. 131; Boye Petersen 1915, p. 293; 1928 I, p. 380; Beger 1927, p. 397, 399.

Regarded by Beger (l. c.) as a bryophilous species of the xerotic type. Is met with in Denmark and Iceland on soil of different kinds (e. g. in a culture of garden soil, Amager), but also on rocks (Iceland), and on thatched roofs (Denmark). In Iceland it is further rather common in fresh water.

Euterrestrial — hydrophytic.

Achnanthes lanceolata Bréb. Cleve Syn. II, p. 191; Van Heurck Syn. pl. 27, fig. 8—11; Østrup 1910, p. 132; Boye Petersen 1915, p. 294; 1928 I, p. 380; *Microneis l.* Beger 1927, p. 401.

Mainly a hydrophytic species, which, however, appears in the hydroterrestrial community. By Beger it is strangely enough referred to the bryophilous species of the xerotic type.

Hydrophytic — hydroterrestrial.

— minutissima Kütz. Cleve Syn. II, p. 188; Van Heurck Syn. pl. 27, fig. 35—38; Østrup 1910, p. 129; Francé 1913, p. 17; Beger 1927, p. 397; Boye Petersen 1928 I, p. 379; 1928 II, p. 14; *A. linearis* Boye Petersen 1915, p. 294.

Thrives quite predominantly in freshwater, but now and then it occurs on the soil. Beger (1927) regards it as bryophilous species of the xerotic type, and Francé (l. c.) mentions it from a pseudoaërial locality. It is hardly possible to keep var. *cryptocephala* apart from the species.

Hydrophytic — pseudoaërial.

3. NAVICULACEAE

I. Amphora.

Amphora Normani Rabh. Cleve Syn. II, p. 119; Østrup 1910, p. 113; Boye Petersen 1915, p. 293; Beger 1927, p. 401; *A. humicola* van Heurck Syn. pl. 1, fig. 12.

The species is evidently not very common, but almost always found on the ground, though also on damp walls, and on bricks which project a little from the soil surface.

Euterrestrial — pseudoaërial.

II. Caloneis.

Caloneis fasciata (Lagst.) Cleve emend. Cleve Syn. I, p. 50; Østrup 1910, p. 11; Boye Petersen 1928 I, p. 382; *Navicula fontinalis* Boye Petersen 1915, p. 286, fig. 4.

Taken in the same extension as done by Cleve, this species contains a very great number of forms. It may well be doubted whether they all belong to the same species. However, in my opinion Hustedt (1930, p. 236, 316) goes too far, when he separates the linear forms provided with distinct apical lines (= var. *typica* Boye P. 1928 I, p. 382) under the name of *Caloneis bacillum* (Grun.) Mereschk. from the smaller and slightly rostrate forms without distinct apical lines, which are referred to the genus *Pinnularia* under the name of *P. fasciata* (Lagst.). For the present I will abide by the limitation of the species by Cleve.

I have (1928 I, p. 382) tried to clear up the division of the species into subspecies.

In reality *Navicula fontinalis* Boye P. comprises several forms of the species, as I have ascertained in revising my preparations. The figured specimen nearly agrees with var. *lenticularis* Østr., but in the preparation forms agreeing with f. *acuta* Boye P. (1928 I, p. 384, fig. 10) are also met with.

The species is hardly distinctly terrestrial; in Iceland it occurs mainly as a hydroterrestrial and pseudoaërial species; in Denmark I have found it growing euterrestrially on cultivated soil. It is not improbable that the different forms will prove to be different in a biological respect; but so far the necessary data for unravelling this are lacking.

III. Frustulia.

Frustulia vitrea Østr. Østrup 1901, p. 262, fig. 30; Boye Petersen 1932 I, p. 17, fig. 3; *Navicula festiva* Krasske 1925, p. 47, taf. I, fig. 16; *N. vitrea* Hustedt 1930, p. 289, fig. 489; *Neidium affine* var. *amphirhyncus* f. *minor* Boye Petersen 1915, p. 286; *Navicula sp.* Boye Petersen 1928 II, p. 16, fig. 3.

It is not improbable that *Navicula hyalina* var. *minima* Bristol (1920, p. 68, fig. 9, 18—21) is identical with the species under consideration. The investigation of this species is so difficult because of its smallness and its very indistinct structure, that a determination of it will always cause trouble.

Is in Denmark met with in Sphagnum tufts in the spring-moor in Hammer Bakker, and in Melby Overdrev on damp soil. Grows only in localities where no lime or salt is present.

Mainly hydroterrestrial.

IV. Navicula.

Naviculae mesolejae.

Navicula Borrichii Boye P. Boye Petersen 1915, p. 285, fig. 1, 2; 1932 I, p. 18.

Occurs on garden soil and on arable land (Sealand, Bornholm). Is also met with on bare sandy soil (Hammer Bakker). Developed in cultures of soil samples from the Botanical Garden, and Amager (garden soil). Only known from Denmark. Has survived drought for 30 months.

Euterrestrial.

— — Boye P. var. **subcapitata** Boye P. Boye Petersen 1915, p. 285, fig. 3; 1928 I, p. 388; 1928 II, p. 15.

Known from Denmark and Iceland. Grows on the surface of arable soil (Bornholm), on uncultivated soil and soil near a house (Iceland).

Euterrestrial.

— — Boye P. var. **undulata** Boye P.; Boye Petersen 1928 I, p. 388, fig. 15; *Stauroneis agrestis* var. Boye Petersen 1915, p. 289.

Known from uncultivated soil (Jutland and Iceland).

Euterrestrial.

— **mutica** Kütz. Cleve Syn. I, p. 129; van Heurck Syn. pl. 10, fig. 17—19; Østrup 1910, p. 37; Francé 1913, p. 19; Boye Petersen 1915, p. 287; Bristol 1920, p. 68; Moore and Carter 1926, p. 118; Bristol Roach 1927 I, p. 569, 577; Beger 1927, p. 401; 1928, p. 383; Boye Petersen 1928 I, p. 389; 1928 II, p. 16; 1932 I, p. 19.

As often pointed out, this species is one of the most wide-spread soil

Diatoms. It is likewise known for its great variability. I have previously (1928 I, p. 389) given an account of its numerous forms. So far nobody has succeeded in finding ecological differences between them with certainty; they are often encountered in company with each other. The species appears to be particularly common on cultivated soil, but on the other hand it is also found on uncultivated soil. Known as a common soil alga from Denmark, England, Germany, America, and East Greenland. Moore and Carter (l. c.) have recorded it from a depth of 4 feet. Beger (l. c.) has found it very commonly in moss tufts and classes it among the constant species of the xerotic type.

Various forms have survived drought for 30—36 months.

Euterrestrial.

The following forms of the species are met with in Denmark:

Navicula mutica Kütz. f. **Cohnii** Hilse. Cleve Syn I, p. 129; Van Heurck Syn. pl. 10, fig. 17; Østrup 1910, p. 37; Boye Petersen 1915, p. 287; 1928 I, p. 391; 1932 I, p. 19.

— — f. **elliptica** Boye P. Boye Petersen 1915, p. 286, fig. 5.

Only known from moss on rocks (Bornholm). Hence perhaps pseudoaërial.

— — f. **Goeppertiana** Bleisch. Cleve l. c.; Van Heurck l. c. fig. 18, 19; Boye Petersen 1915, p. 287; 1932 I, p. 19.

— — f. **minima** Boye P. Boye Petersen 1915, p. 287, fig. 6; 1928 I, p. 392; 1928 II, p. 16; 1932 I, p. 19.

— — f. **quinquenodis** (Grun.) Boye P. Bristol 1920, p. 68; Boye Petersen 1928 I, p. 392; *N. nivalis* Boye Petersen 1915, p. 288; Østrup 1910, p. 37; Cleve Syn. p. 130.

— — f. **rhomboidea** Playfair. Boye Petersen 1928 I, p. 392, fig. 18; 1932 I, p. 19.

— — f. **ventricosa** (Kütz.) Grun. Cleve Syn. I, p. 129; Van Heurck Syn. pl. 4, fig. 1 b; Boye Petersen 1915, p. 288; 1928 I, p. 391.

— **thermicola** Boye P. Boye Petersen 1928 I, p. 394; *N. contempta* Krasske 1929, p. 354, fig. 17; Hustedt 1930, p. 307.

This little species is originally described from Iceland, where it was found on a mossgrown stone and on soil near a hot spring. Later it was found in Germany and described anew by Krasske. The identity of the specimens from Iceland and Germany has been verified by Krasske.

Stauroneis montana Krasske (Hustedt 1930, p. 259, fig. 418) is very like *N. thermicola*.

In Denmark found in soil from the surface (Ermelunden, Amager (garden soil)).

Euterrestrial?

Naviculae entolejae.

Navicula brekkaensis Boye P. Boye Petersen 1928 I, p. 389, fig. 16; 1932 I, p. 19.

In Denmark met with only in the preserved area of Hammer Bakker. Also known from Iceland.

Euterrestrial.

Navicula contenta Grun. Cleve Syn. p. 39; Van Heurck Syn. pl. 14, fig. 31 a (*N. trinodis*); Østrup 1910, p. 39; Boye Petersen 1915, p. 288; Beger 1927, p. 397: 1928, p. 402; Boye Petersen 1928 I, p. 395; 1928 II, p. 15.

Apparently this species attains its full development among mosses, on rocks, and on purely mineral ground; on the other hand the presence of plenty of organic matter seems to all appearance to check its growth.

In Denmark it is found on rocks (Bornholm), but also on the soil of stone-fences and slopes, in most cases in company with f. *biceps* Arnott (Van Heurck Syn. pl. 14, fig. 31 b). This form has by Bristol (1920, p. 70) been found rather commonly in arable soil in England. Forma *parallela* Boye P. (1928 II, p. 15, fig. 2) is likewise met with in Denmark (Hammer Bakker, Boye Petersen 1932 I, p. 19).

Euterrestrial — pseudoaërial.

Naviculæ decipientes.

Navicula gibbula Cleve var. **terrestris** (Boye P.) Boye P. *N. terrestris* Boye Petersen 1915, p. 288, fig. 7, 8; *N. gibbula* Hustedt 1930, p. 285; ? *N. terricola* Bristol 1920, p. 67, fig. 15—17.

Hustedt (l. c.) has pointed out that *N. terrestris* Boye P. in all probability is connected with *N. gibbula* Cleve. In the main it evidently resembles this species, in particular the form described and figured by Lagerstedt (1873, p. 31, pl. I, fig. 7 a) under the name of *N. gibberula*. But it is much smaller, and somewhat finer striped. Hence I am of opinion that it is best kept as a separate variety. I have formerly (1928 I, p. 397) mentioned that *Navicula terricola* Bristol is probably the same form.

In my cultures I have found specimens larger, as well as smaller, than mentioned in the original diagnosis. The limits of dimensions may now be stated as follows: length 15—37 μ, breadth 5—8.5 μ, stripes in the middle 18, towards the apices 24 in 10 μ.

This species is very characteristic also in the girdle view. The corners of the frustule are rounded, the end nodules pointed and distinctly projecting inwards, and the girdle is striped all over with the exception of a very narrow apical area in the middle.

The variety is only known from Denmark and Iceland. In Denmark it is rather common in cultivated soil (Sealand, Bornholm), but was also met with in forest soil (Sealand), and on an uncultivated slope (Bornholm). In Iceland found on purely mineral ground in a couple of localities. Provided that *N. terricola* Bristol prove itself to be the same form, it is also known from Finland, Norway, Hungary, apparently mainly in mineral soils (Fehér 1933, p. 168).

In garden soil (Amager) met with down to 10 cm depth.

Euterrestrial.

Naviculae minusculae.

Navicula Atomus (Näg.) Grun. Cleve Syn. II, p. 4; Van Heurck Syn. pl. 14, fig. 24, 25; Østrup 1910, p. 68; Francé 1913, p. 19; Boye Petersen 1915, p. 289, fig. 11; Bristol 1920, p. 42; Bristol Roach 1927 I, p. 569, 574; Beger 1927, p. 400; 1928, p. 390; Boye Petersen 1928 I, p. 387; 1928 II, p. 15; 1932 I, p. 18; Gistl 1932, p. 647 f; 1933, p. 354.

Comprises several forms, large oval as well as more linear and small forms, of which the structure is difficult to see, and which are only with difficulty distinguished from *N. Vaucheriae* and *N. muralis.*

The species is very common in cultivated (and manured) soil in Denmark; it is recorded in a living state down to 30 cm depth. Has survived drought for 36 months. It is found in other countries under similar circumstances (England, Iceland, Germany, East Greenland).

Euterrestrial.

Navicula muralis Grun. Cleve Syn. II, p. 3; Van Heurck Syn. pl. 14, fig. 26—28; *N. pelliculosa* Boye Petersen 1915, p. 290, fig. 12.

I have revised the very small and hyaline *Navicula*, which I (l. c.) have referred to *N. pelliculosa.* A comparison with Van Heurck Types No. 144 has shown that it is more probably a small form of *N. muralis* Grun.: the somewhat more silicified frustules, the form of the valve, and the smaller size are characters distinguishing it from *N. pelliculosa.* Known as a soil alga only from Denmark (cultivated soil).

Mainly hydrophytic.

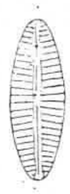

Fig. 5. Navicula Vaucheriae Boye P. (× 1700).

— **Vaucheriae** Boye P. Boye Petersen 1915, p. 291, fig. 13. Fig. nostra 5.

The original description of this species has the defect that the stripes are stated and figured as running strictly perpendicular to the median line; in reality they are very faintly radiating, as can be seen in fig. 5. This figure is drawn from the original specimen. As previously (1915 l. c.) mentioned, the species is much like *N. muralis* Grun. (Van Heurck Types 144, Van Heurck Syn. pl. 14, figs. 26—28); but the stripes are considerably coarser. The form of the valve particularly recalls *N. muralis* f. *sublanceolata* (Van Heurck Syn. pl. 14, fig. 28).

It is hardly possible to distinguish small specimens of *N. Atomus, N. muralis*, and *N. Vaucheriae* from each other. *N. insociabilis* Krasske (1932, p. 114, taf. III, fig. 17) in several respects resembles *N. Vaucheriae;* however, it is very much coarser, with distinctly punctate or transverse striate stripes. By the courtesy of Herr Krasske I have been able to examine the original specimen of this species.

N. Vaucheriae is in the first place met with on garden soil among Vaucheria (Storehedinge). Later I have found it in a similar locality near Gammelkøgegaard, and finally in a culture of a soil sample from Ermelundsletten (surface). In the same place there occurred a more linear form with more distinctly radiating stripes, approaching to *N. Atomus.* The species only met with in Denmark.

Euterrestrial.

Naviculae lineolatae.

Navicula cincta (Ehr.) Kütz. var. **Heufleri** Grun. Cleve Syn. II, p. 16; Van Heurck Syn. pl. 7, fig. 12, 15; Østrup 1910, p. 75; Boye Petersen 1915, p. 291, fig. 14; 1928 I, p. 398.

In reality I am not sure that this variety is not so different from the species that it would be better to regard it as a distinct species. In Denmark it is rather common on cultivated soil. In Iceland it is found on purely mineral ground. Has tolerated drought for 1 month.

Euterrestrial.

f. **curta** Boye P. 1915, p. 291, fig. 15, is often met with in company with the typical var. *Heufleri.*

— **cryptocephala** Kütz. var. **veneta** Kütz. Cleve Syn. II, p. 14; Van Heurck Syn. pl. 8, fig. 3, 4; pl. 14, fig. 34; Østrup 1910, p. 77; Boye Petersen 1915, p. 291; 1928 I, p. 399; 1928 II, p. 16; Gistl 1932, p. 648; 1933, p. 354, 366.

Several forms of *N. cryptocephala* are common in fresh water. Apparently var. *veneta* occurs most frequently on land; nevertheless it is hardly a very pronounced terrestrial form. In Denmark met with on garden soil and meadow soil; in Iceland on damp soil. Gistl (l. c.) has found it mainly in manured soil down to 6 cm depth.

Euterrestrial — hydroterrestrial.

V. Pinnularia.

Pinnularia borealis Ehrb. Cleve Syn. II, p. 80; Van Heurck Syn. pl. 6, fig. 3, 4; Østrup 1910, p. 100; Francé 1913, p. 18; Boye Petersen 1915, p. 292; Bristol 1920, p. 65; Beger 1927; 1928; Boye Petersen 1928 I, p. 401; 1928 II, p. 16; 1932 I, p. 20; Gistl 1933; Fehér 1933, p. 168.

This species was known already at the time of Ehrenberg (1843, 1854) as a soil inhabiting Diatom. On its variation and habitat I have written 1928 I, p. 401. It is one of the commonest soil Diatoms, but notably it is found in abundance on thatched roofs and among mosses (Beger l. c.). Hence it can perhaps really be regarded as a bryophilous species.

Has so far never been met with in the lower layers of the soil.

Among the localities investigated in this work it was only met with in the surface layer on pasture in Holmsland, and garden soil (Amager).

Euterrestrial.

The following 2 forms are recorded from Denmark:

f. **typica** Boye P. Boye Petersen 1928 I, p. 402; 1932 I, p. 20.

f. **subcapitata** Boye P. Boye Petersen 1928 I, p. 402, fig. 23 a; 1928 II, p. 16; 1932 I, p. 20.

— **Brebissonii** Kütz. Cleve Syn. II, p. 78; Østrup 1910, p. 97; Boye Petersen 1915, p. 292; Bristol 1920, p. 66; Boye Petersen 1928 I, p. 403; 1928 II, p. 17; Beger 1928, p. 397, 401; Fehér 1933, p. 168.

Hydrophytic species, which according to my experience is only exceptionally met with on soil. Beger refers it to the bryophilous Diatoms of the hydrotic type.

Hydrophytic — hydroterrestrial.

Pinnularia intermedia Lagst. Lagerstedt 1873, p. 23, tab. I, fig. 3; Østrup 1910, p. 100; Boye Petersen 1915, p. 293, fig. 18, 19; 1928 I, p. 403, fig. 24 a—f (nec f. 6); 1932 I, p. 21; Fehér 1933, p. 168.

I have attempted (1928 I, l. c.) to give a general view of the various forms of the species. Apparently it is more common in northern countries, but seems to occur more sparsely in Denmark. In the localities treated in this work I have not seen it. Besides the localities mentioned in Boye Petersen 1915, l. c. the species is only known in Denmark from Hammer Bakker (the Molinia-bog). Fehér (l. c.) has found it in forest soils from Hungary, Sweden, Finland, and Norway.

Euterrestrial.

— **microstauron** Ehrb. Cleve Syn. II, p. 77; Østrup 1910, p. 95; Boye Petersen 1915, p. 293.

As a soil alga only known from a single locality in Denmark (Melby Overdrev).

Hydrophytic — hydroterrestrial.

— **molaris** Grun. Cleve Syn. II, p. 74; Østrup 1910, p. 93; Boye Petersen 1928 I, p. 406, fig. 26; 1928 II, p. 17.

From Denmark only known as a soil alga from Ermelundsletten, where it occurred at the surface. Apparently more common in Iceland on soil.

Euterrestrial?

— **obscura** Krasske; Krasske 1932, p. 117, fig. 17; A. Schmidt Atl. taf. 388, fig. 18—21; *Pinnularia intermedia* Bristol 1920, p. 66, fig. 8 (6—9); *P. i.* f. 6 Boye Petersen 1928 I, p. 404, fig. 24 g; 1932 I, p. 21. Fig. nostra 6.

1928, l. c. I have described a form which I referred to *P. intermedia* as f. 6. A similar form has previously been figured by Bristol (l. c.) under the name of *P. intermedia*. Meanwhile Krasske (l. c.) has described a new species, *P. obscura*, which quite agrees with my *P. intermedia* f. 6, and because this form has a separate position between the forms of *P. intermedia*, I may follow him in referring f. 6 to the new species. Krasske has found the species in a pseudoaërial locality and in moss tufts (hence presumably euterrestrial). In Iceland I have likewise found it in a pseudoaërial locality. In Denmark I have found it growing euterrestrially (Holmsland, Ermelunden, Botanical Garden, Attrupgaard).

Euterrestrial — pseudoaërial.

— **silvatica** n. sp. *Navicula falaisensis* Boye Petersen 1915, p. 292. Fig. nostra 7.

Valva lineari vel lineari-lanceolata, sæpe apicibus protractis, long. 18—22 μ, lat. 3.8—4.2 μ, striis 24 in 10 μ in media parte radiantibus, ad apices convergentibus. Area centrali rotundata vel late lanceolata, interdum in fasciam transapicalem dilatata.

This species I have (l. c.) mentioned as *Navicula falaisensis;* but a revision of the preparation in question has shown that it must be referred to the genus *Pinnularia,* approaching to *P. appendiculata.* However, it appears to me so different from this species that it is best regarded as a separate species. Notably the stripes are finer, and the cell form is also deviating.

The species has only been observed in a soil sample from dense spruce wood (Rude Skov), and for the present it must therefore be regarded as a euterrestrial form.

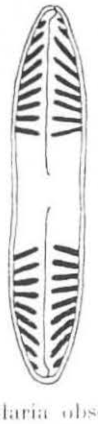

Fig. 6. Pinnularia obscura Krasske. (×1700).

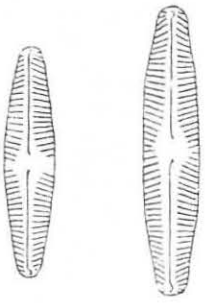

Fig. 7. Pinnularia silvatica n. sp. (×1700).

Pinnularia subcapitata Greg. Cleve Syn. II, p. 75; Hustedt 1930, p. 317, fig. 571, 572; Ostrup 1910, p. 97; Boye Petersen 1915, p. 293; 1928 I, p. 409, fig. 30; 1928 II, p. 17; 1932 I, p. 22.

Known as a soil alga from Iceland and Denmark; however, it is not uncommon in fresh water.

Hydrophytic — euterrestrial?

VI. Stauroneis.

Stauroneis aërophila Boye P. Boye Petersen 1915, p. 289, fig. 10.

Only known from arable soil near Silkeborg.

Euterrestrial?

— **agrestis** Boye P. Boye Petersen 1915, p. 289, fig. 9.

Only known from arable soil near Sveibæk.

Euterrestrial?

— **anceps** Ehrb. Hustedt 1930, p. 256, fig. 405.

A small form of this species developed in cultures of soil samples from East Greenland (surface). In addition the species is of common occurrence in fresh water.

Hydrophytic — pseudoaërial?

4. EPITHEMIACEAE

I. Denticula.

Denticula subtilis Grun. Van Heurck Traité, p. 352, tab. XI, fig. 464; Østrup 1910, p. 201; Boye Petersen 1915, p. 296.

Altogether not a common species. Is presumably best regarded as hydroterrestrial (halophilous).

5. NITZSCHIACEAE

I. Hantzschia.

Hantzschia amphioxys (Ehrb.) Grun. Grunow 1884, p. 47; Francé 1913, p. 20; Boye Petersen 1915, p. 294; Moore and Karrer 1919; Bristol 1920, p. 42; Moore and Carter 1926, p. 118; Bristol Roach 1927 I, p. 569; Beger 1927, p. 397; 1928, p. 390 f.; Boye Petersen 1928 I, p. 414; 1928 II, p. 15; 1932 I, p. 23; Gistl 1932, p. 647 f.; 1933, p. 354–364; Fehér 1933, p. 168.

The forms found on the ground are all small and were previously referred by me to two varieties viz. var. *xerophila* Grun. and var. *genuina* Grun. Between these there is only a difference in size. It would certainly be better to classify the terrestrial forms in a different manner, using the forms of the apices, as I have previously suggested (B. P. 1928 I, p. 414). So far I have not been able to work out such distinctions.

The species comprises marked hydrophytic as well as euterrestrial forms. It is probable that in reality the latter are so different from the former that it would be most correct to regard them as distinct species. Studies on the variation of the cells in single cell cultures will probably be able to yield essential details concerning the constancy of the different forms.

The species is no doubt the most wide-spread soil Diatom, being found in the soil in all parts of the world (Ehrenberg 1854). In Denmark it is exceedingly common and is particularly met with in abundance on cultivated soil, and also, but more sparsely, on uncultivated soil.

It is mainly found in epiterraneous situations, yet on pasture, Holmsland, down to 5 cm depth, and in garden soil (Amager) down to 30 cm depth.

Has survived drought for 36 months. Moore and Karrer (l. c.) have found it down to 1 m depth in America, but it was not particularly common in the samples. On the other hand, Bristol Roach has found it very commonly in arable soils from England from the top layer down to 12 inches' depth. Apparently it thrives best in well manured soil (Gistl l. c., Bristol Roach 1920).

The soil inhabiting small forms of the species must be regarded as euterrestrial.

II. Nitzschia.

Nitzschia communis Rabh. Hustedt 1930, p. 417, fig. 798; Cleve und Grunow 1880, p. 97; Boye Petersen 1928 I, p. 416; *N. Palea* Boye Petersen 1915, p. 295.

Found in great number on garden soil (Sealand). A specimen showed the following dimensions: length 20 μ, breadth 4.7 μ, str. 30—36 in 10 μ, carinal dots 10—12 in 10 μ. These dimensions about agree with *N. Palea* as well as with *N. communis*. The form of the valve, with broadly rounded apices, agrees best with *N. communis*, and therefore I am of opinion that the specimens found must be referred to this species. According to Hustedt (l. c.) the species is common in fresh water. Østrup, on the contrary, (1910, p. 162) has only found the species in a single sample from brackish water. Everything suggests that the two authors must hold different views of the species.

Hydrophytic — euterrestrial?

— — var. **abbreviata** Grun. Cleve und Grunow 1880, p. 97; Boye Petersen 1915, p. 294; 1928 I, p. 416; 1932 I, p. 24.

Met with in Denmark in a meadow near Lejre (Sealand), and in Hammer Bakker on mud in the spring moor (hydrophytic).

In Iceland common on the soil on manured spots.

Hydrophytic — hydroterrestrial?

— **debilis** (Arnott) Grun. Cleve und Grunow 1880, p. 68; Hustedt 1930, p. 400, fig. 759 (*N. tryblionella* var. *debilis*); Østrup 1910, p. 146; Boye Petersen 1915, p. 294; 1928 I, p. 416; 1932 I, p. 24; Beger 1927, p. 401.

This small hyaline species has no doubt often been overlooked. It does not seem to be particularly common on soil. Recorded as a soil inhabitant from Denmark, Iceland, and Germany.

Presumably mostly hydroterrestrial.

— **dissipata** (Kütz.) Grun. Cleve und Grunow 1880, p. 90; Hustedt 1930, p. 412, fig. 789; Østrup 1910, p. 155; Boye Petersen 1928 I, p. 416; Beger 1927, p. 401; *N. lanceolata* var. *incrustans* Boye Petersen 1915, p. 295.

On revising my preparation of 1915, which according to my determination at the time was to contain *N. lanceolata* var. *incrustans*, I found but few Nitzschia frustules nearly all seen in girdle view. Only a single frustule was seen in valve view. It showed the following dimensions: Length 23.4 μ, breadth 3.5 μ, striae very fine, carinal dots 8—9 in 10 μ, carina somewhat excentric. It is evident, then, that the frustules found must be referred to *N. dissipata*.

This species is not unknown as an inhabitant of rocks; but it is evidently most widespread in fresh water.

In Denmark and in Iceland I have found it on soil, and Beger has found it in some few localities in Germany among mosses.

Hydrophytic — pseudoaërial.

— **Hantzschiana** Rabh. Cleve und Grunow 1880, p. 99; Hustedt 1930, p. 415, fig. 797; Østrup 1910, p. 163; Boye Petersen 1915, p. 295; 1928 I, p. 417.

Only found a few times on soil; in Denmark in meadow and on sandy soil (Sealand, Moen); in Iceland among mosses on a stone near a therm.

Hydrophytic — hydroterrestrial.

Nitzschia inconspicua Grun. Cleve und Grunow 1880, p. 99; Hustedt 1930, p. 415 (*N. Frustulum* var. *perpusilla*); Østrup 1910, p. 163; Boye Petersen 1915, p. 295; Bristol 1920, p. 71, fig. 10. 3—6; Beger 1927, p. 401.

It is no doubt questionable whether the small Nitzschia forms referred by Bristol and myself to this species are really identical. For the present, however, I am not able to give any better determination of them. In Denmark I have found such forms on garden soil (Sealand) while Bristol records similar finds from England in 6 different samples of arable soil.

Hydrophytic — euterrestrial?

— **Kützingiana** Hilse. Cleve und Grunow 1880, p. 96; Hustedt 1930, p. 416, fig. 802; Østrup 1910, p. 164; Boye Petersen 1915, p. 295; Moore and Karrer 1919, p. 304; Boye Petersen 1928 I, p. 417; 1928 II, p. 16; 1932 I, p. 24; Gistl 1933, p. 369; Singh 1933, p. 107.

As shown by the above citations, the species has often been mentioned among terrestrial Diatoms. On account of its hyaline frustules it may easily be overlooked. Has been found on the surface of the soil as well as in the lower layers.

Hydrophytic — euterrestrial.

— — Hilse f. **terrestris** Boye P. Boye Petersen 1932 II, p. 407, fig. 14, 15.

Only known from Denmark. Pasture on Holmsland. Found there at 10—20 cm depth; and from garden soil, Amager, at the same depth.

Euterrestrial?

CHLOROPHYCEAE

I. Euchlorophyceae.

1. VOLVOCACEAE

I. Carteria.

Carteria sp.

A *Carteria* species occurred in a culture of a soil sample from East Greenland (40 cm depth).

II. Chlamydomonas.

Chlamydomonas fusiformis Boye P. Boye Petersen 1932 II, p. 396, fig. 1—3; Gistl 1932, p. 644; 1933, p. 354.

The species has been described from a culture of sand from the surface in a dune depression on Holmslands Klit. Gistl also thinks he has found it in cultures from soil samples in Germany.

Euterrestrial.

— **terrestris** Boye P. Boye Petersen 1932 I, p. 28.

The species only known from Denmark. Besides in the original locality, Hammer Bakker, where it was evidently very widespread, I have since seen it in cultures of soil samples from Ermelunden and Amager (garden soil), in both places at depths from 0—30 cm.

Euterrestrial.

Chlamydomonas sp.

Species of *Chlamydomonas* which it was not possible to identify, occurred in several of the soils examined, from the surface down to a depth of 30 cm. The localities were Holmsland, Ermelunden, Ermelundsletten, Botanical Garden, Amager.

2. CHLOROSPHÆRACEAE

I. Fernandinella.

Fernandinella alpina Chod. Chodat 1921, p. 106, 114, fig. 19, 20; Boye Petersen 1928 II, p. 19; 1932 I, p. 34, fig. 16.

Only known from Switzerland, Iceland and Denmark. Has survived drought for 36 months. Danish localities: Hammer Bakker, the Botanical Garden, Ermelundsletten, Amager.

Euterrestrial.

3. CHLOROCOCCACEAE

I. Chlorococcum.

Chlorococcum humicola (Näg.) Rabh. ?Francé 1913, p. 22; Bristol 1919 I, p. 473, p. 17, 18 (partim?); 1919 II, p. 99; 1920, p. 75; Moore and Carter 1926, p. 119; Boye Petersen 1932 I, p. 30, fig. 12, 13; Gistl 1932, p. 647; 1933, p. 354 ; Fehér 1933, p. 172; *Cystococcus humicola* Nägeli 1849, taf. II, fig. 26; *Protoderma viride* Moore and Karrer 1919, p. 304 (fide Moore and Carter l. c.).

It has previously been questioned by me (l. c.) whether the alga mentioned by Bristol (l. c.) is really identical with *Cystococcus humicola* Näg.; for the same reasons as previously adduced I will not alter the name here either. All the modern authors cited above presumably hold the same view of the species as Bristol.

In England, Germany, America, and Denmark *C. humicola* has proved to be one of the commonest soil algae. It has been found on the surface as well as down to a depth of 5 feet 5 inches (Moore and Carter l. c.). In my experiments it survived drought for 36 months. Found by Bristol (1919 II, p. 99) in soil samples up to 60 years old. In Denmark I have found it at depths from 0 to 30 cm.

Danish localities: Hammer Bakker, the Botanical Garden, Holmsland, Holmslands Klit (arable, dune depression), Borris Heath, Ermelunden, Ermelundsletten, Amager (garden soil).

Euterrestrial.

II. Dictyococcus.

Dictyococcus irregularis Boye P. Boye Petersen 1932 II, p. 400, fig. 4—8.

Only known from Denmark. Localities: Holmslands Klit (arable, 5 cm depth), same locality (dune depression, surface).

Euterrestrial.

Dictyococcus minor (Schmidle) Petrová; Petrová 1931, p. 221; Boye Petersen 1932 II, p. 402; *Botrydiopsis minor* Chodat 1913, p. 174, fig. 147, 148; Boye Petersen 1928 II, p. 18; 1932 I, p. 26.

In mixed alga cultures this species will easily be mistaken for certain stages of *Heterococcus viridis*. It is recognisable, however, by the dark green chromatophores and the distinct cellulose reaction of the cell walls with chlor-zinc-iodine.

I have found it as a soil alga in Iceland, in East Greenland, and in Denmark. Here I have seen it at depths from 0—30 cm. Has survived drought for up to 68 weeks.

Danish localities: Hammer Bakker, Holmsland, Holmslands Klit (arable), Borris Heath, Ermelunden, Ermelundsletten, Botanical Garden, Amager.

Euterrestrial?

— **sp.**

In a culture from the soil surface in Ermelunden there occurred a Dictyococcus sp., which seemed to deviate somewhat from the two abovementioned species.

III. Macrochloris.

Macrochloris dissecta Korsh. Korshikov 1926, p. 476; Printz 1927, p. 451, fig. 362; Boye Petersen 1932 I, p. 33, fig. 15.

Originally Korshikov found this species in fresh water. In Denmark, however, it is not uncommon in soil.

Danish localities: Hammer Bakker, Holmsland, Holmslands Klit (dune depression), Botanical Garden, Amager.

Euterrestrial — hydrophytic.

4. OOCYSTACEAE

I. Chlorella.

Chlorella botryoides Boye P. Boye Petersen 1932 I, p. 36, fig. 18.

The species only known from Denmark. Found originally in cultures of soil samples from Hammer Bakker. Later finds: Holmslands Klit (arable, 0—10 cm depth), Borris Heath (surface), Botanical Garden (surface).

Euterrestrial.

— **ellipsoidea** Gern. Gerneck 1907, p. 250, taf. 11, fig. 45—51; Boye Petersen 1915, p. 331, fig. 27—30; 1928 I, p. 431.

It has previously been supposed by myself and other investigators (Puymaly 1924, p. 207) that the ellipsoidal Chlorella forms growing on palings and tree-trunks were identical with *C. ellipsoidea* Gern. Now, however, I have my doubts about this. Gerneck's species has been described from cultures the material of which was derived from small pools, and we are, then, perhaps confronted with a hydrophytic species, which resembles the aërial forms in appearance, but cannot be regarded as the same species, since they probably differ physiologically. Only pure cultures can furnish further information on this point.

In the surface of the soil at Ermelundsletten I have seen cells that entirely agreed with *C. ellipsoidea* Gern. Whether these cells perhaps represent a third, euterrestrial form, or perhaps one of the other two, must be left undecided.

Chlorella vulgaris Beyerinck; Beyerinck 1890, p. 730; Bristol 1927 I, p. 569; Gistl 1932, p. 647 f.; 1933, p. 355; Fehér 1933, p. 170.

It is presumably more than doubtful whether all species mentioned in the literature (also in the more recent works) as *Chlorella vulgaris* do indeed rightly bear this name. But as a rule it is quite impossible to verify, since no details are available. It is highly probable that pretty different species lurk under this name. *Chlorella vulgaris* may then be regarded as a species collectiva.

Danish localities: Ermelunden, Ermelundsletten (0—10 cm depth), Botanical Garden, Amager (garden soil, 0—10 cm depth).

Euterrestrial — hydrophytic.

— — var.? Boye Petersen 1932 I, p. 36, fig. 17.

From Hammer Bakker and from pasture on Holmsland I have seen a form which would appear to deviate somewhat from the species. It is distinguished especially by its distinct pyrenoids and the often numerous autospores within each mother cell.

Euterrestrial.

II. Muriella.

Muriella terrestris Boye P. Boye Petersen 1932 II, p. 403, fig. 9.

The species is very common in all kinds of soil in Denmark down to a depth of 30 cm. Found also in East Greenland in the surface and at 30 cm depth. Does not form macroscopically visible growths on the surface, but often develops in cultures; in Bristol's liquid medium, however, it does not grow quickly, so it will often be outgrown by other species. Has survived drought for 36 months.

Danish localities: Holmslands Klit (arable land), Ermelundsletten, Ermelunden, Botanical Garden, Amager.

Euterrestrial.

5. COELASTRACEAE

I. Dictyosphærium.

Dictyosphærium minutum Boye P. Boye Petersen 1932 I, p. 37, fig. 19.

Only known from Denmark; has survived drought for 8 months.

Localities: Hammer Bakker, Botanical Garden, Ermelunden.

Euterrestrial.

II. Keratococcus.

Keratococcus bicaudatus (A. Braun) Boye P. Boye Petersen 1928 I, p. 429; 1928 II, p. 19; 1932 I, p. 37; *Dactylococcus bicaudatus* A. Braun in Rabh. Fl. Eur. alg. Sect. III, 1868, p. 47; Boye Petersen 1915, p. 332; Bristol 1920, p. 74; 1927 I, p. 569; *Keratococcus caudatus* Gistl 1933, p. 355.

I have previously accounted for the synonymy of the species (1928 I, p. 429). It is known as a soil alga from Germany, England, Iceland, East Greenland, Denmark; but it mostly grows on other substrata, such as stumps of trees, old Polypori, thatched roofs etc.

Known on soil in Denmark from the following localities: Bornholm, Moen, Sealand, Ermelundssletten, Hammer Bakker.

Aëroterrestrial.

III. Scenedesmus.

Scenedesmus tetradesmiformis (Wolz.) Chod. Chodat 1926, p. 141; Boye Petersen 1932 I, p. 38, fig. 20.

Occurred in a culture of a soil sample from Hammer Bakker. Since otherwise the species occurs in the plankton, it must be regarded as hydroterrestrial — hydrophytic.

IV. Coccomyxa.

Coccomyxa dispar Schmidle; Schmidle 1901, p. 20, tab. I, fig. 6—25; Boye Petersen 1915, p. 323; 1928 I, p. 424; Fehér 1933, p. 172.

The species is of common occurrence as macroscopically visible slimy layers on moss, stumps of trees and the like, and likewise on soil. Fehér (l. c.) states that he has found it in 50 p. c. of the samples of forest soil examined by him. It did not develop in any of my cultures of soil samples.

Only recorded from Denmark as growing on soil (raw humus) on a wayside slope in Ry Norreskov.

Possibly aëroterrestrial or pseudoaërial.

— **Naegeliana** (Artari) Wille; Wille 1911, p. 38. Boye Petersen 1915, p. 323; 1932 I, p. 32; Jaag 1933 I, p. 63; *Gloeocystis Naegeliana* Artari 1892, p. 235, pl. 6, fig. 17—39; *Byssus botryoides* Müller 1767, p. 219?; Fl. dan. 1782, tab. 899, 3; *Palmella botryoides* Lyngbye 1819, p. 205; Hornemann 1837, p. 624 ("klaseformet Sliimpunct").

It has been questioned by Pascher (Lemmermann, Brunnthaler und Pascher 1915, p. 210) whether this species can rightly be referred to the genus *Coccomyxa*. In his monograph on the genus *Coccomyxa* (1933 I, p. 63) Jaag has, however, admitted the close affinity of the species to *C. dispar*, so he does not insist that it should be omitted from the genus. I have previously (1928 I, p. 423) pointed out the great similarity between these two species and have especially emphasised that in practice the presence or absence of pyrenoids is often very difficult to establish with certainty. I have also thought (1915, p. 323) that *C. subellipsoidea* Acton was the same species. This is disputed by Jaag (1933 I, p. 64), who maintains that the alga in *Botrydina* never has pyrenoids. I have not investigated this more closely as I have had no living material; but I have previously repeatedly found *Botrydina* growing in company with *C. Naegeliana* (1915, p. 324). Hence I should not be surprised if there existed *Botrydina* forms containing an alga furnished with pyrenoid. This has already previously been observed by Acton (1909 I and II). Since she also ascribes multiplication by zoospores to the alga, her observations were early doubted (Lemmermann, Brunnthaler und Pascher 1915, p. 210), and Jaag (1933 I, p. 116, II, p. 184) thinks that her *C. subellipsoidea* comprises two species, viz. a *Coccomyxa* without pyrenoids and a *Chlamydomonas* with pyrenoids. But there is the further possibility that the *Coccomyxa*, too, may have contained pyrenoids and therefore have been closely related to or identical with *C. Naegeliana*. Hence I do not consider it absolutely certain that *C. subellipsoidea* Jaag (1933 I, p. 121, II, p. 181) is identical with *C. subellipsoidea* Acton.

It is, indeed, not improbable that the earlier authors by *Byssus botryoides* meant especially *Botrydina vulgaris* (Fl. dan. l. c.), since, as mentioned above, this species is often seen in company with *Coccomyxa Naegeliana*.

In Lyngbye's herbarium there are 3 capsules containing *Palmella botryoides*, two of which are from the Faeroes, corresponding to those mentioned in the "Hydrophytologia", viz.:

1) "21 Juny 1817 in terra humida umbrosa ad Thorshavn", to which is added "in peninsula Hals Fioniae etiam legi". This sample contains *Coccomyxa Naegeliana*; the cells are oblong, with pyrenoids, length 6–8 μ, breadth 3–4 μ, embedded in a mucous mass, often 2 or 4 together. The sample further contains *Botrydina vulgaris*.

2) "7 Sept. 1817 on Sphagn. obtusifolium in umbrosis west of Thorshavn."

There is something similar in this, but the mucous sheaths are somewhat less coherent.

3) "On horse chestnuts growing in the shade near Hofmansgave 21 Feb. 1815": contains a piece of bark with *Desmococcus* (*Pleurococcus*) *vulgaris* (Näg.) Brand?, whereas I could detect nothing resembling *Palmella botryoides*. It is possible that this species is so sparsely represented that it has escaped my attention.

From the available data it must be assumed that by the name *Palmella botryoides*, Lyngbye meant *Coccomyxa Naegeliana*.

The species has been found in Denmark on soil (as a rule raw humus) in Bornholm, but in addition on stumps of trees, tree-trunks, rocks, and on Sphagnum in Magle Mose in Grib Skov.

Mainly aëroterrestrial — pseudoaërial.

Coccomyxa sp.?

In a culture from Borris Heath (0—5 cm depth) there occurred a form with elongated cells embedded in mucilage.

6. ULOTHRICACEAE

I. Geminella.

Geminella terricola Boye P. Boye Petersen 1932 I, p. 39, fig. 21.

It now seems doubtful to me whether this form can rightly be referred to the genus *Geminella*. It is quite possible that it should in reality be regarded as a *Gloeotila* form of *Hormidium flaccidum*. In the cultures established by me for the present work, I have had but slight occasion to observe it. I have thus been obliged to postpone a determination of its true nature until I have a better opportunity to examine it. Only in a soil sample from the Botanical Garden which had been left dry for 12 months have I seen anything resembling it.

Euterrestrial?

II. Hormidium.

Hormidium flaccidum (Kütz.) A. Br. sensu lato; Klebs 1896, p. 341; Boye Petersen 1915, p. 338; Piercy 1917; Boye Petersen 1928 I, p. 432; 1928 II, p. 19; 1932 I, p. 40; Fehér 1933, p. 172; Gistl 1933, p. 355 f.; *Ulothrix flaccida* Kützing 1849, p. 349; *Stichococcus flaccidus* Gay 1891, p. 79.

Under the name of *Hormidium flaccidum* I have here gathered together several nearly related forms, which it is difficult to keep distinct in enrichment cultures. On the other hand, it is probable that by observing them in pure cultures, it will in future be possible to recognise them in enrichment cultures, too.

Under the name of *Ulothrix* species several authors have mentioned various forms of Ulothricaceae which can hardly by the means now available be kept distinct from *H. flaccidum* in the limitation here introduced. I may mention *Ulothrix subtilis* var. *variabilis* (Bristol 1920, p. 76, 1927 I, p. 569), *U. subtilis*, *U. tenerrima* (Gistl 1932, p. 647), *U. subtilis* (Moore and Carter 1926), *U. variabilis* (Moore and Karrer 1919).

If we include these, too, under *Hormidium flaccidum*, this species has a wide distribution as a soil alga, and it may also occur on other substrata such as the bark of trees, rocks, on mosses, and perhaps in water, too. Much evidence, however, goes to show that the species comprises several physiologically, and hence presumably also biologically different forms. Thus the *Hormidium* described by Piercy (l. c.) does not behave exactly like the one mentioned by Klebs (l. c.) and Münster Strøm has shown (1928) that different strains of *Hormidium* behaved differently with respect to the pH value of the substratum. For the present it is impossible for us to take these facts into consideration in our determination of the Hormidia occurring in nature, hence I have been obliged to use the above-mentioned very wide delimitation of the species.

Known as a common soil alga in England, Germany, America, Iceland, East Greenland and Denmark. In general found only on the surface: Moore and Karrer alone (1919) state their *U. variabilis* to be derived from a depth of 60 cm. In this connection I may remind the reader that in my seepage experiments (see above p. 100) *Hormidium* passed through the column of earth.

Hormidium has survived desiccation in soil for 36 months.

Danish localities: Bornholm, Moen, Sealand, Holmsland, Holmslands Klit (arable), Ermelundsletten, Botanical Garden, Amager.

Aëroterrestrial — euterrestrial.

Hormidium mucosum Boye P. Boye Petersen 1915, p. 340, tab. III, fig. 38—40; *Hormidium flaccidum* Puymaly 1924, p. 224 (partim).

Puymaly (l. c.) claims to have established that such forms as I have described under the name of *H. mucosum* are merely dry soil forms of *H. flaccidum*. I am not quite convinced, however, that he is right; I have not since had an opportunity of verifying his investigations, so for the present I will maintain the species. It is known, then, from France and Denmark. Found exclusively on soil of different kinds: clay, sand, boggy soil, and raw humus (Sealand, Bornholm).

Euterrestrial.

III. Rhaphidonema.

Rhaphidonema sempervirens Chod. Chodat 1913, p. 165, fig. 141—143; Boye Petersen 1928 II, p. 19.

The genus *Rhaphidonema* was established by Lagerheim as the name of an alga derived from red snow; later on Chodat referred to the genus the present species which he found in water in the environs of Geneva. I have seen a form agreeing with Chodat's description in cultures of soil samples from Iceland.

Not previously recorded from Denmark.

Danish locality: Ermelundsletten (10 cm depth).

Hydrophytic — euterrestrial.

IV. Stichococcus.

Stichococcus bacillaris Näg. Nägeli 1849, p. 76, tab. 4 G; Francé 1913, p. 13; Boye Petersen 1915, p. 336; Moore and Karrer 1919, p. 304; Bristol 1920, p. 76; Moore and Carter 1926, p. 122; Bristol Roach 1927 I, p. 569; Boye Petersen 1928 I, p. 433; 1932 I, p. 41; Gistl 1933, p. 368.

The species is presumably most widely distributed as a eu-aërial alga, since it grows on all kinds of prominent objects. Still, it has also early been found on soil as well as subterraneously at barely 1 metre's depth (Moore and Carter l. c.). Known as a soil alga from Germany, England, America, Denmark, and East Greenland. Has survived drought in my experiments for 8 months. According to Bristol (1919 II, p. 99) it has kept alive in soil samples up to 48 years.

Danish localities: Tokkekob Hegn, Asserbo Plantage, Ermelundsletten, Botanical Garden.

Aëroterrestrial.

— **exiguus** Gern. Gerneck 1907, p. 262, taf. 12, fig. 66—76; Boye Petersen 1932 I, p. 41.

Known in Denmark from Hammer Bakker only. The species originally cultivated by Gerneck, but he does not say whence he derived the material for his cultures.

Euterrestrial?

7. BLASTOSPORACEAE

I. Prasiola.

Prasiola crispa (Lightfoot) Menegh. Meneghini 1838, p. 36; Boye Petersen 1915, p. 346; 1928 I, p. 433; *Muscus uvida amans* etc. P. Kylling 1688, p. 105; *Oscillatoria muralis* Lyngbye 1819, p. 95; Fl. dan. 1818, tab. 1599, 2; *Prasiola muralis* Boye Petersen 1915, p. 346; *Schizogonium crispum* Gay 1891, p. 86; *S. murale* Gay 1891, p. 87; *Ulva crispa* Fl. dan. 1828, tab. 1840; Hornemann 1837, p. 707; *U. terrestris* Lyngbye 1819, p. 32; Hornemann 1837, p. 707; Liebmann 1839, p. 472.

In the Hormidium form the species is very common in Denmark on the lower part of tree-trunks, and in favourable circumstances it will grow far up them; in other cases it spreads on the ground at the foot of the tree. Very common, too, on thatch.

In the Prasiola form, from the frequent mention made of it in the earlier literature, it must be presumed to have been more common previously than now. From our day I have only seen it in one sample from Denmark, viz. from Charlottenlund (1934 leg. K. Gram). It may very well be supposed that owing to more sanitary conditions it will nowadays more rarely find sufficiently favourable circumstances to develop the leaf form. This form seems only to appear when it is abundantly provided with manures. In Iceland I have seen it very commonly developed as Prasiola around the farmsteads near liquid manure and the like.

I have previously mentioned the specimens at hand from Denmark (1915 l. c.).

Aëroterrestrial.

8. VAUCHERIACEAE

I. Vaucheria.

Vaucheria hamata Walz; Walz 1866, p. 156, fig. 12—17; Heering 1907, p. 159, fig. 84; Boye Petersen 1915, p. 349; Bristol 1920, p. 75; Boye Petersen 1928 I, p. 437.

The earlier descriptions of *Vaucheria* species were very imperfect (Vaucher sub *Ectosperma*); Walz (1866) recognised the impossibility of identifying the species with certainty from the descriptions. He then adopted the method of discarding entirely a number of the earlier descriptions and describing a number of new species, without any pedantic consideration for the fact that they were likely to have been described before, though badly. Unfortunately Walz chose as names for his "new" species earlier names of discarded species of Vaucheria. This was undoubtedly a mistake which contributed to create further confusion. Nevertheless I am of opinion that for the present we must abide by Walz's designations, since we are here on firm ground owing to his excellent descriptions of the species. Only if a study of the herbarium material left by the earlier authors should show that it can be established with certainty what the author in question really meant by his descriptions, will it be necessary to discard Walz's species and substitute the original, earlier names for his.

Thus I have tried by means of Herbarium Lyngbye to clear up what Lyngbye meant by his species. However, I have not been successful in all cases, thus not in the case of *V. caespitosa* Lyngb. As the locality of this species Lyngbye gives "terra uda argillacea ad Körup Fioniæ", and the original specimen is available, I have examined it, but have not been able to identify the species with certainty. The same capsule, however, contains a specimen labelled by Lyngbye "Ectosperma cæspitosa d. 29. Maj 1816 in fossa in Riisagrene pr. Hofmansgave"; in it were found oogonia in pairs measuring 65 μ in length, 51 μ in breadth, and altogether corresponding closely to *V. hamata* Walz.

Further, Herbarium Lyngbye contained under *V. Dillwynii* a capsule labelled as follows: "Ectospermum Dillwynii, Ceramium Dillwynii in umbrosis horti botanici Hafniensis. NB. Sub microscopico vidi d. 2 May 1816, et omnino referebat Ectospermum cæspitosum Vaucher". On the inner side of the capsule there is a label from which it appears that the sample was sent by Hornemann to Hofman-Bang with the determination *E. Dillwynii*. Lyngbye later re-identified it as *E. caespitosum*. The sample undoubtedly contained *V. hamata* Walz.

It is then certain that by *V. caespitosa*, Lyngbye meant, at any rate partly, *V. hamata* Walz. Since, however, the original specimen of *V. caespitosa* Lyngb. does not seem to be this species, there is no sufficient reason for discarding Walz's name.

The species is that most commonly occurring in Denmark on garden soil, arable land, and meadow soil (Sealand, Funen, Bornholm). Its further distribution is mentioned in Heering 1907, p. 160.

Euterrestrial.

Vaucheria sessilis (Vauch.) D. C. f. **repens** Rabh. Heering 1907, p. 144, fig. 68; *V. repens* Boye Petersen 1915, p. 349.

This species is presumably not common as a soil alga in Denmark: only observed once on garden soil (Sealand). Its further distribution is mentioned by Heering (l. c.).

Hydroterrestrial.

— **terrestris** (Vauch.) Lyngbye; Lyngbye 1819, p. 77, tab. 21; Hornemann 1837, p. 699; Heering 1907, p. 160, fig. 85; Boye Petersen 1915, p. 349; 1928 I, p. 138; *V. hamata* Lyngbye 1819, p. 77, tab. 20, fig. C; Fl. dan. 1821, tab. 1726, 1; Hornemann 1837, p. 698; ?*V. frigida* Hornemann 1837, p. 700; *Ectosperma terrestris* Vaucher 1803, p. 27; *E. hamata* Vaucher 1803, p. 26.

The original specimen of *V. hamata* Lyngbye is present in Herb. Lyngbye. It bears the label: "16 April 1816 in stagnosis Körup Fioniæ" and thus corresponds to the specimen cited in the Hydrophytology. It is finely fructifying. Oogonia measured 116—154 μ in length, 98—121 μ in breadth; they were seated singly above the antheridium and curved in the same plane with the latter. I can therefore confirm De Wildeman's assertion (1896, pp. 79, 81) that *V. hamata* Lyngbye = *V. terrestris*. By *Vaucheria hamata* both Lyngbye and Vaucher presumably meant the hydrophytic form of *V. terrestris*.

The species is not particularly common on soil in Denmark. Recorded from soil in meadows and pastures (Sealand, Fiunen). Its general distribution is given in Heering (1907).

Hydrophytic — hydroterrestrial.

II. Conjugatae.

1. DESMIDIACEAE

I. Cosmarium.

Cosmarium sp.

In a culture of a soil sample from East Greenland a *Cosmarium* grew up which was not more closely identified.

II. Cylindrocystis.

Cylindrocystis Brebissonii Menegh. W. and G. S. West Monogr. I, p. 58, pl. IV, fig. 23—32, pl. V, fig. 10; Jacobsen 1875—76, p. 165;. Boye Petersen 1915, p. 315; 1928 II, p. 19; Fehér 1933, p. 170.

This species did not occur in any of my cultures of soil samples; on the other hand Fehér (l. c.) has found it in 10 p. c. of the forest soils examined by him, while I myself have previously found it in a culture of a soil sample from Iceland.

It can hardly grow on soil except where there is a supply of terrestrial water. In Denmark recorded only as a soil alga from Bornholm.

Pseudoaërial.

— — var. **minor** W. and G. S. West Monogr. I, p. 59, pl. 5, fig. 11; Boye Petersen 1915, p. 315.

Occurs in similar places as the species. In Denmark known only from Bornholm as a soil alga.

Pseudoaërial.

III. Mesotaenium.

Mesotaenium macrococcum (Kütz.) Roy et Biss. var. **micrococcum** (Kütz.) W. and G. S. West; W. and G. S. West Monogr. I, p. 52, pl. 4, fig. 1—3; Boye Petersen 1915, p. 314.

The present species as well as the species and forms of the genus Mesotaenium mentioned in the sequel are especially characteristic of "dripping rocks" and similar localities.

The species is only known from one locality in Denmark, viz. Ambjerg (Sealand).

Pseudoaërial.

— **chlamydosporum** de Bary; W. and G. S. West Monogr. I, p. 52, pl. 4, fig. 4—14; Boye Petersen 1915, p. 314; 1932 I, p. 25.

As a soil alga known only from Bornholm and Hammer Bakker.

Pseudoaërial.

— — f. **minor** W. and G. S. West Monogr. I, p. 53. Boye Petersen 1932 I, p. 26.

Only found in Hammer Bakker in company with the species.

Pseudoaërial.

Mesotaenium violascens de Bary; W. and G. S. West Monogr. I, p. 55, pl. 3, fig. 27—33; Boye Petersen 1915, p. 315; 1932 I, p. 26. Fehér 1933, p. 170.

I have generally found this species and not the other species of the genus on heath, whereas Graebner (1895, p. 548) mentions *M. Braunii* (= *M. macrococcum*) as common on the heaths of northern Germany. Whether this determination is correct, and accordingly another species prevails in northern Germany than in Denmark must be left undecided. Fehér (l. c.) found *M. violascens* in 55 p. c. of the forest localities examined.

Danish localities: Borris Heath, Bornholm, Hammer Bakker.

Pseudoaërial — euterrestrial.

2. ZYGNEMATACEAE

I. Zygogonium.

Zygogonium ericetorum (Roth) Kütz. Kützing 1843, p. 92; Warming 1906, p. 90, 119, 161; Boye Petersen 1915, p. 315; 1928 I, p. 420; 1932 I, p. 26; Czurda 1932, p. 105, 222; Gau 1934; *Conferva ericetorum* Schumacher 1803, p. 107; Fl. dan. 1816, tab. 1548, 2; Lyngbye 1819, p. 140, tab. 47; Hornemann 1837, p. 659.

Though this species has repeatedly been subjected to pretty thorough investigations (West and Starkey 1915, Hodgetts 1918, Fritsch 1916, Czurda 1931, Gau 1934), its morphology and systematic position cannot be said to be entirely cleared up. Czurda alleges (1932 l. c.) that the forms mentioned in the litérature can develop under special external conditions from various different species. This is disputed by Gau (l. c.). For the present, therefore, *Zygogonium ericetorum* must be maintained as a species; but it will no doubt be necessary to study it more closely.

From Denmark the species is known from old times as growing on acid, peaty soil (Jutland, Sealand, Bornholm), principally in places which are flooded in the winter but dry in the summer.

Hydroterrestrial.

III. Heterokontae.

1. CHLOROTHECIACEAE

I. Characiopsis.

Characiopsis Heeringiana Pascher; Pascher 1925, p. 63, fig. 44 a; Boye Petersen 1932 I, p. 27, fig. 8.

Known from 2 localities in Denmark, viz. Hammer Bakker, Holmslands Klit (5 cm depth).

Euterrestrial?

— **minuta** Borzì; Pascher 1925, p. 62, fig. 43a; Heering 1906, p. 101, fig. 7a, b; Moore and Carter 1926, p. 123; Boye Petersen 1932 I, p. 27, fig. 9.

Not known from Denmark as a soil alga, having only been observed in a culture of mud from the spring moor in Hammer Bakker. On the other hand, it is recorded from America (Moore and Carter l. c.) as occurring at a depth of 2 feet 3 inches in the soil.

Hydroterrestrial?

2. OPHIOCYTIACEAE

I. Bumilleriopsis.

Bumilleriopsis brevis (Gern.) Printz; Printz 1914, p. 50, tab. 4, fig. 192—198; Boye Petersen 1928 I, p. 421, fig. 32, 33; 1928 II, p. 18; *Ophiocytium breve* Gerneck 1907, p. 241, tab. 11, fig. 24—30.

Not previously known from Denmark. Met with mainly on the surface and on one occasion down to a depth of 5 cm. Has survived drought for 9 months.

Danish localities: Holmsland, Ermelundsletten, Botanical Garden, Amager.

Euterrestrial.

3. CHLOROBOTRYDACEAE

I. Monodus.

Monodus acuminatus (Gern.) Chod. Chodat 1913, p. 185; Boye Petersen 1932 I, p. 28; *Chlorella acuminata* Gerneck 1907, p. 249, taf. 11, fig. 37—44; Boye Petersen 1928 II, p. 18.

In Denmark only known from Hammer Bakker: found in addition in Germany and Iceland.

Euterrestrial or hydroterrestrial.

— **subterraneus** Boye P. Boye Petersen 1932 II, p. 406, fig. 13.

Only known from Denmark. Localities: Ermelundsletten (surface), Holmslands Klit (arable, 10 cm depth).

Euterrestrial.

II. Pleurochloris.

Pleurochloris magna Boye P. Boye Petersen 1932 II, p. 403, fig. 10—12.

Only known from Denmark. Localities: Holmsland (pasture, 10—30 cm depth), Holmslands Klit (arable, 0—30 cm depth), ibidem (dune depression, 5 cm depth).

Euterrestrial.

— **sp.**

Forms referable to this genus have been found in the following localities: Ermelunden (surface), Botanical Garden, and in East Greenland (surface).

Possibly they might be referred to *Pleurochloris commutata* Pascher. That species is mentioned as a soil alga by Gistl 1933, pp. 355, 356, and by Fehér 1933, p. 172.

4. TRIBONEMACEAE

I. Bumilleria.

Bumilleria exilis Klebs. Klebs 1896, p. 389, taf. II, fig. 15—20; Bristol 1920, p. 78; 1927 I, p. 569, 576; Boye Petersen 1928 I, p. 421; 1928 II, p. 18; 1932 I, p. 26, fig. 6; Gistl 1933, p. 355 f.

The species was first described by Klebs from cultures to which clayey soil had been added. It has turned out to be common in soil in Iceland, England, East Greenland, and Denmark. Here I have realised it in cultures of very different soils. Survived drought for 36 months. In England it has been shown to be present down to a depth of 12 in. and in Denmark down to 30 cm. (Botanical Garden, Amager).

Localities in Denmark: Hammer Bakker, Holmsland, Ermelunden, Ermelundsletten, Botanical Garden, Amager.

Euterrestrial.

— **sicula** Borzi; Borzi 1895, p. 186, tav. 16, 17; Bristol 1927 I, p. 569, 576; Boye Petersen 1932 I, p. 27, fig. 7.

The species is evidently much rarer than the preceding one. In Denmark only known from Hammer Bakker and the Botanical Garden. Has survived drought for 30 months.

Euterrestrial.

II. Tribonema.

Tribonema bombycinum (Ag.) Derb. et Sol. Pascher 1925, p. 101; Boye Petersen 1915, p. 313: Bristol 1920, p. 78: 1927 I, p. 569: Boye Petersen 1928 II, p. 20.

The species hardly grows frequently on or in soil; but germs of it are evidently occasionally found in the earth and develop in moist periods, e. g. in puddles.

Danish localities: Ledreborg (forest road), Ermelunden (0—20 cm depth), Ermelundsletten (surface).

Hydroterrestrial — hydrophytic.

III. Heterococcus.

Heterococcus viridis (Gern.) Chod. Chodat 1908, p. 80; 1909, p. 74, pl. 5, 6; Bristol 1927 I, p. 569, 576; Boye Petersen 1928 II, p. 19; *Monocilia viridis* Gerneck 1907, p. 263; Moore and Carter 1926, p. 122.

Known from England as well as from America, Iceland, and East Greenland as a common soil alga: in America down to a depth of 3 $^1/_2$ feet, in East Greenland down to 40 cm. Not previously recorded from Denmark; it is quite possible, however, that a number of the forms which I have previously referred to *Botrydiopsis minor* have really been forms of *Heterococcus*. Has survived drought for 36 months.

Danish localities: Botanical Garden (0—30 cm depth), Ermelunden (0—20 cm), Ermelundsletten (0—30 cm), Amager (garden soil, 0—30 cm.).

Euterrestrial.

5. BOTRYDIACEAE

I. Botrydium.

Botrydium granulatum (L.) Grev. Klebs 1896, p. 223, taf. 1, fig. 17—19; Pascher 1925, p. 117, fig. 95; *Linckia granulata* Schumacher 1803, p. 114; *Ulva granulata* O. Fr. Müller 1767, p. 217; Fl. dan. 1777, tab. 705; *Vaucheria granulata* Lyngbye 1819, p. 78.

Though this species has been known in Denmark since the time of O. Fr. Müller (1767), only a few collections of it are available. Thus there are no specimens of it in Herbarium Lyngbye. Lyngbye's description of its growth (l. c.) is, however, so apposite that quite obviously he has seen the plant himself. The Danish localities which can be established as certain are: Wallensgaards Mose, Bornholm 1827 (Hornemann scr.), Hvalso (Sealand 1881 L. Kolderup Rosenvinge), Hjortekæret, Dyrehaven 1934 (K. Gram), Damhussoen 1934 (K. Gram), the bog in the Botanical Garden 1934 (J. B. P.), Olsted near Horsens, garden walk 1934 (S. Lund).

The species is probably in reality very common in certain localities, viz. in the dried up mud in ditches, pools, round lakes etc. Has also been met with on garden walks in the autumn. Further, it requires a certain degree of dryness in order to attain full development, therefore it is only seen in brief periods.

Hydroterrestrial.

RHODOPHYCEAE

1. BANGIACEAE

I. Porphyridium.

Porphyridium cruentum Näg. Nägeli 1849, p. 000 taf. 00.

Of this species only one sample collected in Denmark is found in the Botanical Museum, viz. from the Cactus house in the Botanical Garden (J. B. P. 1934). Otherwise recorded as growing on soil wetted with urine, hence it is presumably highly nitrophilous.

Euterrestrial.

VIII. Summary.

1. In the introduction it is attempted to give to the term "soil algae" a more precise delimitation, so as to make it comprise the species that normally occur on and in the soil.

2. A brief account is given of the development of our knowledge concerning soil algae through the ages.

3. The discovery of the subterranean alga flora at the beginning of the 20th century is passed in review.

4. It is shown through the writer's investigations that in Danish soils, too, a subterranean alga flora is met with, on cultivated as well as on uncultivated land.

The investigations were chiefly made in the spring, and it then turned out that the number of algal germs was generally much higher at the surface than in the lower layers. The algal species of the deeper layers are the same as are met with on the surface. This has been found, too, by previous investigators.

5. The investigation of a single locality in East Greenland shows that subterranean algae are present also in the absolutely virgin soil there.

6. A series of investigations, in part published before, show that soil algae do not multiply appreciably in soil samples in the dark; this has been found for Diatoms, Cyanophyceae, and Chlorophyceae. In similar soil samples there was, on the other hand, a vigorous growth of the algae in the light.

7. The writer, therefore, could not but suppose (as surmised by some of the previous authors too) that the subterranean algae are derived from the surface of the soil and thence carried down into the lower layers.

8. After passing in review the literature on the responses of the motile phases of the algae, the writer arrives at the result that an active descent of the algae is not probable. The writer's own experiments gave negative results in all cases.

9. On the other hand, it was probable at the outset that seeping water, especially during heavy showers after a prolonged drought, might carry a large number of germs down into the soil.

Experiments showed that water dripped on to the surface of a column of earth with an intensity as during a heavy shower is capable of carrying algal germs through the column of earth. Algae of all the three systematic main groups were thus passed through a column of earth 12—15 cm deep.

10. It turned out that the algae passed more readily through soil which contained earthworms; whereas none of the writer's experiments showed that earthworms alone were able to convey algae into the soil.

11. BRISTOL (1919 II) has found living algal germs in soil samples up to 73 years old. These contained 10—3 p.c. of water. The writer carried out experiments in a somewhat different way, letting soil samples dry up in the course of about a month until they contained only about 1.5 p.c. of water. The experiment would seem to show that during the first time a not inconsiderable number of algal cells perish; but thereupon a state supervenes in

which the number of living algal germs and the number of species remain constant for several years. A desiccation so intense and prolonged as that of the experiment will hardly occur under natural conditions in this country.

12. The data from the literature would seem to show that the effect of frosts or heat also will not be able to kill all the algae at the surface of the soil, though a smaller or larger number of them will perish.

13. It is pointed out that we know very little as to the relation which the acidity of the soil bears to the algae.

14. Other conditions being equal, the content of nutrient substances in the soil seems to determine the luxuriance of the algal vegetation. According to the data, manured soils possess a much richer algal flora than unmanured land.

15. A review of the literature on the capacity of algae to fix nitrogen gives as a result that such a capacity has not been shown to be present in any Chlorophyceae or Diatoms. Among the Cyanophyceae species of *Anabaena* and *Nostoc* seem to be capable of binding free N in the light, but not in darkness.

For Chlorophyceae as well as for Cyanophyceae there is much evidence to show that they can stimulate the activity of nitrogen-fixing bacteria. All the evidence would also seem to indicate that the algae can only exercise this influence in the light. The writer therefore arrives at the conclusion that only the epiterranean algae bear any relation to the fixation of nitrogen, while the subterranean algae are without importance in this respect.

16. In respect of the importance of the soil algae for the soil a sharp distinction must, according to BRISTOL ROACH (1927 I), be drawn between the epiterranean and the subterranean algae, since they must exercise contrary influences. Epiterranean algae are producers, subterranean algae consumers.

The number of algae at the surface of the soil may under favourable circumstances be so high that they must be assumed to be capable of exerting their influence side by side with the bacteria. The number of the subterranean algae is always, as far as is known, so low that their importance in relation to the bacteria will be minimal.

The significance of the epiterranean algae for the carbon cycle in the soil must be found in the fact that they produce organic substances by photosynthetic assimilation of carbon dioxide (preparation of "new soil"). In the nitrogen cycle most of them appear to play a part by stimulating the activity of nitrogen-fixing bacteria. Only some few genera of Cyanophyceae seem to be capable of assimilating free N in the light. According to the data at hand, the subterranean algae are exclusively consumers and bear no essential relation either to the carbon or the nitrogen cycle.

17. A list is given of the Danish soil alga flora as also of the algae met with in East Greenland, with systematic and biological notes on each species.

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LITERATURE CITED

Acton, E. 1909 I. Coccomyxa subellipsoidea, a new member of the Palmellaceae. Ann. of Bot. **23:** 573.

— 1909 II, Botrydina vulgaris Brébisson, a primitive Lichen. Ann. of Bot. **23:** 579.

Aderhold, R. 1888. Beitrag zur Kenntnis richtender Kräfte bei der Bewegung niederer Organismen. Jenaische Zeitschr. f. Naturw. **22:** 310.

Agardh, C. A. 1812, Dispositio Algarum Sueciæ, Lundæ.

— 1817, Synopsis Algarum Scandinaviæ, Lundæ.

Allison and Morris 1930 I, Nitrogen fixation by blue-green Algæ. Science **71:** 221.

— 1930 II, Nitrogen-fixation by soil algae. Proc. and Papers Second intern. Congr. Soil Sci. III Comm. 1932.

Artari, A. 1892, Untersuchungen über Entwicklung und Systematik einiger Protococcoideen. Bull. d. l. soc. imp. des Naturalistes de Moscou, Nouv. sér. **6:** 222.

— 1899, Ueber die Entwicklung der grünen Algen unter Ausschluss der Bedingungen der Kohlensäure-Assimilation. Bull. soc. imp. des naturalistes de Moscou N. S. **14:** 39.

— 1901, Zur Ernährungsphysiologie der grünen Algen. Ber. d. d. bot. Ges. **19:** 7.

— 1902, Über die Bildung des Chlorophylls durch grüne Algen. Ber. d. d. bot. Ges. **20:** 201.

— 1904—1909, Der Einfluss der Nährlösungen auf die Entwicklung einiger grüner Algen I, II, III. Pringsh. Jahrb. f. Wiss. Bot. **40:** 591, **43:** 177, **46:** 443.

— 1913—14, Zur Physiologie der Chlamydomonaden. Pringsh. Jahrb. f. Wiss. Bot. **52:** 410, **53:** 527.

Beger, H. 1927, Beiträge zur Ökologie und Soziologie der luftlebigen Kieselalgen. Ber. d. d. bot. Ges. **45:** 385.

— 1928, Atmophytische Moosdiatomeen in den Alpen. Vierteljahrschr. Naturf. Ges. Zürich **73**, Beibl. **15:** 382.

Benecke, W. 1898, Mechanismus und Biologie des Zerfalles der Conjugatenfäden in die einzelnen Zellen. Pringsh. Jahrb. f. wiss. Bot. **32:** 453.

— 1900, Über farblose Diatomeen der Kieler Föhrde. Pringsh. Jahrb. f. Wiss. Bot. **35:** 567.

Beyerinck, M. W. 1890, Culturversuche mit Zoochlorellen, Lichengonidien und anderen niederen Algen. Bot. Zeit. **48:** 725.

— 1893, Bericht über meine Kulturen niederer Algen auf Nährgelatine. Centralbl. f. Bakt. **13:** 368.

— 1898, Notiz über Pleurococcus vulgaris. Centralbl. f. Bakt. II, **4:** 785.

— 1901, Ueber oligonitrophile Mikroben. Centralbl. f. Bakt. II, **7:** 561.

Bolte, E. 1920, Über die Wirkung von Licht und Kohlensäure auf die Beweglichkeit grüner und farbloser Schwärmzellen. Pringsh. Jahrb. f. wiss. Bot. **59:** 287.

Bonar and Goldsmith 1925, Distribution and behavior of soil algae. Carnegie Inst. Washington Year Book **24:** 324.

Boresch, K. 1913, Die Färbung von Cyanophyceen und Chlorophyceen in ihrer Abhängigkeit vom Stickstoffgehalt des Substrates. Pringsh. Jahrb. f. wiss. Bot. **52:** 145.

Bornebusch, C. H. 1930, The fauna of forest soil. Copenhagen.

Bornet et Flahault 1886—1888, Revision des Nostocacées Hétérocystées. Ann. des Sc. nat. Sér. VII, Bot. T. III, IV, V, VII.

Borzì, A. 1895, Studi algologici II. Palermo.

Bouilhac, R. 1896, Sur la fixation de l'azote atmosphérique par l'association des algues et des bactéries. Compt. rend. acad. Paris **123:** 828.

— 1897, Sur la culture du Nostoc punctiforme en présence du glucose. C. R. Acad. des sc. Paris **125:** 880.

— 1898 I, Recherches sur la végétation de quelques algues d'eau douce. Thèses Paris 1898.

— 1898 II, Sur la végétation d'une plante verte, le Nostoc punctiforme à l'obscurité absolue. C. R. Acad. des sc. Paris **126:** 1583.

— 1901, Sur la végétation du Nostoc punctiforme en présence de différents hydrates de carbone. C. R. Acad. des sc. Paris **133:** 55.

Bouilhac et Giustiniani 1903, Sur une Culture de sarrasin en présence d'un mélange d'algues et des bactéries. C. R. Acad. des sc. Paris **137:** 1274.

— 1904, Sur des cultures de diverses plantes supérieures en présence d'un mélange d'algues et des bactéries. C. R. Acad. des sc. Paris **138:** 293.

Braun, A. 1851, Betrachtungen über die Erscheinung der Verjüngung in der Natur. Leipzig.

Brierley, W. B. 1928. The microflora of the soil. Journ. Quekett microsc. club **16:** 9.

Brierley, Jewson and Brierley 1927, The quantitative study of soil Fungi. Proc. and Papers of the First Internat. Congr. of soil sc. III.

Bristol, B. M. 1919 I, On a malay form of Chlorococcum humicola (Näg.) Rabh. Journ. Linn. Soc. **44:** 473.

— 1919 II, On the retention of vitality by algae from old stored soils. New Phytol. **18:** 29.

— 1920, On the algal-flora of some desiccated English soils. Ann. of Bot. **34:** 35.

Bristol Roach, B. M. 1926, On the relation of certain soil algae to some soluble carbon compounds. Ann. of Bot. **40:** 149.

Bristol Roach, B. M. 1927 I, On the algae of some normal English soils. Journ. of Agric. Sc. **17:** 563.

— 1927 II, On the carbon nutrition of some algae isolated from soil. Ann. of Bot. **41:** 509.

— 1927 III, The present position of our knowledge of the distribution and function of algae in the soil. Proc. and Pap. First internat. Congr. soil sc. 1927 **3:** 30.

— 1928 I, Bodenalgen in Abderhalden Handbuch, Abt. XI, Teil 3, p. 747, 811.

— 1928 II, On the Influence of light and of glucose on the growth of a soil alga. Ann. of Bot. **42:** 317.

Bristol and Page 1923, A critical enquiry into the alleged fixation of nitrogen by green algae. Ann. of applied biology **10:** 378.

Brunnthaler, J. 1909, Der Einfluss äusserer Faktoren auf Gloeothece rupestris. Sitz.-ber. Akad. d. Wiss. Wien, Math.-naturw. Kl. **118:** 501.

— 1915, Protococcales in Die Süsswasserflora Deutschlands etc. Heft 5.

Buder, J. 1917, Zur Kenntnis der phototaktischen Richtungsbewegungen. Pringsh. Jahrb. f. wiss. Bot. **58:** 105.

Burkholder, P. R. 1933, Movement in the Cyanophyceae. The effect of pH upon movement in Oscillatoria. Journ. Gen. Physiol. **16:** 875.

Charpentier, P. G. 1903 I, Alimentation azotée d'une algue, le Cystococcus humicola. Ann. Inst. Pasteur **17:** 321.

— 1903 II, Recherches sur la physiologie d'une algue verte. Ann. Inst. Pasteur **17:** 369.

Chmielewsky, V. 1904, Über Phototaxis und die physikalischen Eigenschaften der Kulturtropfen. Beih. z. Bot. Centralbl. **16:** 53.

Chodat, F. 1928 I, Sur la spécifité des Stichococcus du sol du Parc National. C. R. Soc. Phys. et Hist. nat. Genève **45:** 26.

— 1928 II, Note préliminaire sur la flore algologique des sols du parc national. Verh. d. Schweiz. Naturf. Ges. **109:** 191.

— 1931, Études d'algologie du sol. Sur le genre Schizococcus gen. nov. Ber. d. Schweiz. Bot. Ges. **40:** XXIII.

Chodat, R. 1908, Sur un genre nouveau de Conferve. Bull. de l'herb. Boiss. 2. Sér. **8:** 80.

— 1909, Étude critique et expérimentale sur le polymorphisme des algues. Genève.

— 1913, Monographies d'algues en culture pure. Matériaux pour la Flore cryptogamique Suisse **4**.

— 1921, Matériaux pour l'histoire des algues de la Suisse. Bull. d. l. soc. bot. de Genève 2. sér. **13:** 66.

— 1926, Scenedesmus. Rev. d'Hydrologie **3:** 71.

Cholodny, N. 1930, Über eine neue Methode zur Untersuchung der Bodenmikroflora. Arch. f. Mikrobiol. **1:** 620.

Cleve, P. T. 1894—95. Synopsis of the naviculoid Diatoms I, II. Kgl. Sv. Vet.-Akad. Handl. **26:** No. 2; **27:** No. 3.

Cleve und Grunow 1880, Beiträge zur Kenntnis der arctischen Diatomeen. Kgl. Sv. Vet.-Akad. Handl. **17:** No. 2.

Cohn, F. 1854, Untersuchungen über die Entwicklungsgeschichte der mikroskopischen Algen und Pilze. Verh. Leop. Car. **24:** 103.

— 1867, Über die Gesetze der Bewegung der mikroskopischen Pflanzen und Thiere unter dem Einfluss des Lichtes. Bot. Zeitung **25:** 171. (Referat).

Comère, J. 1906, Observations sur la périodicité du développement de la flore algologique dans la région toulousaine. Bull. Soc. bot. de Fr. **53:** 390.

— 1910, De l'évolution périodique des algues d'eau douce dans les formations passagères. Bull. Soc. bot. de Fr. **57:** 558.

— 1913, De l'action du milieu considerée dans ses rapports avec la distribution générale des algues d'eau douce. Mémoires de la Soc. bot. de France Nr. 25.

Conn, H. J. 1918. N. Y. Agric. Exper. Station, Technical Bull. Nr. 64. (not seen).

Czurda, V. 1931, Zur Morphologie und Systematik der Zygnemalen. Beih. z. Bot. Centralbl. **48:** 238.

— 1932, Zygnemales in Die Süsswasserflora Deutschlands etc. Heft 9, 2. Aufl.

Dangeard, A.-P. 1921, Observations sur une algue cultivée à l'obscurité depuis huit ans. C. R. Acad. Sc. Paris **172:** 254.

Deby, J. 1879, Les Diatomées terrestres. Journ. of the Roy. Micr. Soc. **2:** 162 (not seen).

Deherain et Demoussy 1900, Sur la culture des lupins bleus (Lupinus angustifolius). C. R. Acad. sc. Paris **130:** 465.

De Wildeman, É. 1896, Observations sur quelques espèces du genre Vaucheria. Bull. soc. roy. de bot. de Belgique **35:** 71.

Dillenius, J. J. 1741, Historia muscorum. Oxonii.

Dillwyn, L. W. 1809, British Confervae. London.

Dönz, O. Christ. 1934, Chlorella Zofingiensis, eine neue Bodenalge. Ber. d. Schweiz. Bot. Ges. **43:** 127.

Drewes, K. 1928, Über die Assimilation des Luftstickstoffs durch Blaualgen. Centralbl. f. Bakt. II, **76:** 88.

Dusi, H. 1933, Ernährungsphysiologie einiger Euglenen-arten. Ann. Inst. Pasteur.

Ehrenberg, C. G. 1843, Verbreitung und Einfluss des mikroskopischen Lebens in Süd- und Nordamerika. Abh. Berl. Akad.

— 1854, Mikrogeologie. Leipzig.

Engelmann, W. 1882, Über Licht- und Farbenperception niederster Organismen. Pflügers Archiv f. die ges. Phys. **29:** 387.

Esmarch, F. 1911, Beitrag zur Cyanophyceenflora unsrer Kolonien. Jahrb. d. Hamb. wiss. Anst. **28:** 3. Beiheft.

— 1914, Untersuchungen über die Verbreitung der Cyanophyceen auf und in verschiedenen Böden. Hedwigia **55:** 224.

Étard et Bouilhac 1898, Sur la présence de chlorophylle dans une Nostoc cultivée à l'abri de la lumière. C. R. Acad. sc. Paris **127:** 119.

Famintzin, A. 1866, Die Wirkung des Lichtes auf die Bewegung der Chlamydomonas pulvisculus Ehrb., Euglena viridis Ehrb. und Oscillatoria insignis Fw. Bull. Acad. Petersb. **10:** 534.

Famintzin, A. 1867, Die Wirkung des Lichtes auf Algen etc. Pringsh. Jahrb. f. wiss. Bot. **6:** 1.

Fechner, R. 1915, Die Chemotaxis der Oscillarien und ihre Bewegungserscheinungen überhaupt. Zeitschr. f. Bot. **7:** 289.

Fehér, D. 1933, Untersuchungen über die Mirkobiologie des Waldbodens. Berlin.

Fischer, H. 1926, Das Edaphon, ein Sammenbegriff für Biocoenosen von mikroskopischer Wasser- und Mossflora, sowie der Aërophilen. Actes de la IV conférence internat. de Pédologie Rome **3:** 17.

Flora danica 1764—1883. København.

Francé, R. 1913, Das Edaphon. München.

Frank, B. 1888, Untersuchungen über die Ernährung der Pflanze mit Stickstoff und über den Kreislauf desselben in der Landwirtschaft. Landw. Jahrb. **17:** 421.

— 1889, Über den experimentellen Nachweis der Assimilation freien Stickstoffs durch erdbewohnende Algen. Ber. d. d. bot. Ges. **7:** 34.

Frank, Th. 1904, Kultur und chemische Reizerscheinungen der Chlamydomonas tingens. Diss. Basel 1904. Bot. Zeit. **62:** 153.

Fraymouth, J. 1928, The moisture relation of terrestrial algae III. Ann. of Bot. **42:** 75.

Freund, H. 1908, Neue Versuche über die Wirkungen der Aussenwelt auf die ungeschl. Fortpflanzung der Algen. Flora **98:** 41.

Fritsch, F. E. 1907 I, A general consideration of the subaërial and freshwater algal flora of Ceylon. Proc. Roy. Soc. B. **79:** 197.

— 1907 II, The subaërial and freshwater algal flora of the tropics. Ann. of Bot. **21:** 235.

— 1907 III, The rôle of algal growth in the colonization of new ground and in the determination of scenery. The Geographical Journ. **30:** 531.

— 1916, The morphology and ecology of an extreme terrestrial form of Zygnema (Zygogonium) ericetorum (Kütz.) Hansg. Ann. of Bot. **30:** 135.

— 1922 I, The moisture relations of terrestrial algae I. Ann. of Bot. **36:** 1.

— 1922 II, The terrestrial alga. Journ. of Ecology **10:** 220.

Fritsch and Haines 1923, The moisture relations of terrestrial algae. Ann. of Bot. **37:** 683.

Fritsch and Salisbury 1915, Further observations on the heath association on Hinhead common. New Phytologist **14:** 116.

Gau, B. 1934, Beiträge zur Morphologie und Biologie von Zygogonium ericetorum Inaug. Diss. Königsberg.

Gautier et Drouin 1888, Recherches sur la fixation de l'azote par le sol et les végétaux. C. R. Acad. sc. Paris **106:** 754.

— 1891, Recherches sur la fixation de l'azote par le sol arable. C. R. Acad. sc. Paris **113:** 820.

Gay, F. 1891, Recherches sur le développement et la classification de quelques algues vertes. Thèse, Paris.

Geitler, L. 1925, Cyanophyceae in Die Süsswasserflora Deutschlands etc. H. 12.

— 1927, Häutung bei einer pennaten Diatomee. Österr. bot. Zeitschr. **76:** 98.

— 1930—32, Cyanophyceae in Rabenhorst Kryptogamenflora **14**.

Gerneck, R. 1907, Zur Kenntnis der niederen Chlorophyceen. Beih. z. Bot. Centralbl. **21**, II: 221.

Gistl, R. 1932, Zur Kenntnis der Erdalgen. Arch. f. Mikrobiol. **3:** 634.

— 1933, Erdalgen und Düngung. Erdalgen und Anionen. Arch. f. Mikrobiol. **4:** 348.

Glade, R. 1914, Zur Kenntnis der Gattung Cylindrospermum. Cohns Beitr. z. Biol. d. Pfl. **12:** 295.

Gomont, M. 1890, Essai de classification des Nostocacées homocystées Journ. de Bot. **4:** 349.

— 1892, Monographie des Oscillariées. Ann. des sc. nat. sér. VII Bot. Tome 15—16.

— 1899, Sur quelques Oscillariées nouvelles. Bull. d. l. soc. bot. de France **46:** 25.

Graebner, P. 1895, Studien über die norddeutsche Heide. Engl. bot. Jahrb. **20:** 500.

Gregory, W. 1856, Notice of some new species of British fresh-water Diatomaceae. Quarterly Journ. Micr. Sc. **4.**

Grintzesco, J. 1903, Contribution à l'étude des Protococcacées. Chlorella vulgaris. Rev. générale de Bot. **15:** 1.

— 1929, Contribution à l'étude de la microflore des sols de Roumanie. C. R. XIVe Congrès internat. d'agriculture Bucarest.

Grunow, A. 1884, Die Diatomeen von Franz Josefs-Land. Denkschr. d. k. Akad. d. Wiss. Wien, math.-naturw. Kl. **48**, II Abth.

Hansgirg, A. 1882, Neue Beobachtungen über die Bewegungen der Oscillarien. Botan. Centralbl. **12:** 361.

Harder, R. 1917 I, Über die Beziehung des Lichtes zur Keimung von Cyanophyceensporen. Pringsh. Jahrb. f. wiss. Bot. **58:** 237. Ber. d. d. bot. Ges. **35:** (58) 1918.

— 1917 II, Ernährungsphysiologische Untersuchungen an Cyanophyceen. Zeitschr. f. Bot. **9:** 145.

— 1918, Über die Bewegung der Nostocaceen. Zeitschr. f. Bot. **10:** 177.

Hariot, P. 1891, Le genre Polycoccus Kützing. Journ. de Bot. **5:** 29.

Harrison and Subramania Ayier 1913, The gases of swamp rice soils I. Mem. of the Dep. of agric. in India, Pusa Chem. Ser. **3:** 65.

— 1914, The gases of swamp rice soils II. Mem. of the Dep. of agric. in India Pusa Chem. Ser. **4:** 1.

Hassall, A. H. 1845, A History of the British freshwater algae I—II. London.

Heering, W. 1906. Die Süsswasseralgen Schleswig-Holsteins. 1. Heterokontæ. Jahrb. der Hamb. wiss. Anstalten **23:** 61.

— 1907, Die Süsswasseralgen Schleswig-Holsteins. II, Chlorophyceæ. Jahrb. der Hamb. wiss. Anstalten **24:** 103.

— 1914, Ulothricales, Microsporales, Oedogoniales in Die Süsswasserflora Deutschlands etc. Heft 6.

— 1921, Siphonocladiales, Siphonales in Die Süsswasserflora Deutschlands etc. Heft 7.

Hegler, R. 1901, Untersuchungen über die Bau der Phycochromaceenzelle. Pringsh. Jahrb. f. wiss. Bot. **36:** 229.

Heiberg, P. A. C. 1863, Conspectus criticus Diatomacearum Danicarum. København.

Heinze, B. 1906 I, Einige Beiträge zur mikrobiologischen Bodenkunde. Centralbl. f. Bakt. II, **16:** 640.

— 1906 II, Über die Stickstoffassimilation durch niedere Organismen. Landw. Jahrb. **35:** 889.

Hodgetts, W. L. 1918, The conjugation of Zygogonium ericetorum. New Phytologist **17:** 238.

Hoffmann-Grobéty 1912, Contribution à l'étude des algues unicellulaires en culture pure. Bull. Soc. bot. Genève 2 sér. **4:** 73.

Hopkins and **Wann** 1926, Relation of hydrogen-ion-concentration to growth of Chlorella and to availability of iron. Bot. Gaz. **81:** 353.

Hornemann, J. W. 1796, Forsøg til en dansk oekonomisk Plantelære. København.

— 1837, Dansk oekonomisk Plantelære, Anden Deel. København.

Hustedt, F. 1930, Bacillariophyta (Diatomeæ) in Die Süsswasserflora Deutschlands etc. Heft 10, 2. Aufl.

Jaag, O. 1929, Recherches expérimentales sur les gonidies des lichens appartenant aux genres Parmelia et Cladonia. Thèse, Genève.

— 1933 I, Coccomyxa Schmidle, Monographie einer Algengattung. Beitr. z. Kryptogamenfl. der Schweiz.

— 1933 II, Botrydina vulgaris Bréb. Ber. d. Schweiz. Bot. Ges. **42:** 169.

Jacobsen, H. C. 1910, Kulturversuche mit einigen niederen Volvocaceen. Zeitschr. f. Bot. **2:** 145.

— J. P. 1875—76, Aperçu systématique et critique sur les Desmidiacées du Danemark. Bot. Tidsskr. **8:** 143.

Jensen, P. 1893, Über Geotropismus niederer Organismen. Pflügers Archiv f. die ges. Physiologie **53:** 428.

Jones, J. 1930, An investigation into the Bacterial Associations of some Cyanophyceae. Ann. of Bot. **44:** 721.

Kärcher, H. 1931, Kurze Mittheilung. Über die Kälteresistenz einiger Pilze und Algen. Planta **14:** 515.

Karsten, G. 1901, Über farblose Diatomeen. Flora **89:** 404.

— 1928, Bacillariophyta (Diatomeæ) in Engler und Prantl: Die natürlichen Pflanzenfamilien 2. Aufl. **2:** 105.

Klebs, G. 1887, Beiträge zur Physiologie der Pflanzenzelle. Ber. d. d. bot. Ges. **5:** 181.

— 1896, Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena.

Koch und **Kossowitsch** 1893, Über die Assimilation von freiem Stickstoff durch Algen. Bot. Zeit. **51:** 321.

Koffman, M. 1928, Eine Methode zur direkten Untersuchung der Mikrofauna und der Mikroflora des Bodens. Centralbl. f. Bakt. II, **75:** 28.

— 1929, Zur Methode der direkten Untersuchung der Mikrofauna und Mikroflora des Bodens. Centralbl. f. Bakt. II, **78:** 337.

— 1931, Weiteres zur Methode der direkten Untersuchung der Mikrofauna und Mikroflora des Bodens. Centralbl. f. Bakt. II, **85:** 1.

Koffman, M. 1934, Die Mikrofauna des Bodens, ihr Verhältnis zu anderen Mikroorganismen und ihre Rolle bei den mikrobiologischen Vorgängen im Boden. Archiv f. Mikrobiologie **5:** 246.

Kol, E., et Chodat, F. 1934, Quelques algues nouvelles des sols et de la neige du Parc National Suisse, Engadine. Bull. Soc. bot. de Genève 2. Sér. **25:** 250.

Kolbe, R. W. 1932, Grundlinien einer allgemeinen Ökologie der Diatomeen. Ergebnisse der Biologie **8:** 221.

Kolkwitz, R. 1926, Zur Ökologie und Systematik von Botrydium granulatum (L.) Grev. Ber. d. d. bot. Ges. **44:** 533.

Korniloff, M. 1913, Expériences sur les gonidies des Cladonia pyxidata et Cladonia furcata. Bull. Soc. bot. de Genève 2. sér. **5:** 114.

Korshikov, A. A. 1926, On some new organisms from the groups Volvocales and Protococcales etc. Arch. f. Protistenk. **55:** 439.

Kossowitsch, P. 1894, Untersuchungen über die Frage, ob die Algen freien Stickstoff fixieren. Bot. Zeit. **52:** 97.

Krasske, G. 1925, Die Bacillariaceen-Vegetation Niederhessens. Abh. und Bericht 56 des Vereins für Naturk. zu Cassel E. V.

— 1927, Diatomeen deutscher Solquellen und Gradierwerke. Archiv f. Hydrobiologie **18:** 252.

— 1929, Beiträge zur Kenntnis der Diatomeenflora Sachsens. Bot. Arch. **27:** 348.

— 1932, Beiträge zur Kenntnis der Diatomeenflora der Alpen. Hedwigia **72:** 92.

Krüger, W. 1894, Beiträge zur Kenntnis der Organismen des Saftflusses der Laubbäume. Zopfs Beitr. z. Phys. und Morph. niederer Org. **4:** 69.

Krüger und Schneidewind 1900, Sind niedere chlorophyllgrüne Algen imstande den freien Stickstoff der Atmosphære zu assimilieren und den Boden an Stickstoff bereichern? Landw. Jahrb. **29:** 776.

Kufferath, H. 1912, Notes sur la physiologie et la morphologie de Porphyridium cruentum Naeg. Bull. Soc. R. bot. Belgique **52:** 286.

— 1913, Contribution à la physiologie d'un Protococcacée nouvelle. Rec. Inst. Bot. Leo Errera **9:** 113.

— 1920, Observations sur la morphologie et la physiologie de Porphyridium cruentum Naeg. Rec. Inst. Bot. Leo Errera **10:** 1.

— 1929, La culture des algues. Revue algologique **4:** 127.

Kylling, P. 1688, Viridarium danicum. Hafniæ.

Kützing, F. T. 1843, Über die systematische Eintheilung der Algen. Linnæa **17:** 75.

— 1845, Phycologia germanica. Nordhausen.

— 1849, Species algarum. Lipsiæ.

— 1845—71, Tabulæ phycologicæ. Nordhausen.

— 1865, Die kieselschaligen Bacillarien oder Diatomeen. Nordhausen.

Lagerstedt, N. G. W. 1873, Sötvattens-Diatomaceer från Spetsbergen och Beeren Eiland. Bih. till kgl. sv. Vet.-Akad. Handl. **1,** Nr. 14.

Leick, E. 1932, Zur Methodik der relativen Taumessung. Beih. z. Bot. Centralbl. **49,** Ergbd: 160..

Leick, E. 1933, Der Tau als Standortsfaktor. Ber. d. d. bot. Ges. **51:** 409.

Lemmermann, Brunnthaler und Pascher 1915, Chlorophyceæ II in Süsswasserflora Deutschlands etc. Heft 5.

Lidforss, B. 1907, Die wintergrüne Flora. Lunds Univ. Aarsskr. N. F. **2**, Afd. 2, 13.

Liebmann, F. M. 1839, Bemærkninger og Tillæg til den danske Algeflora. Nath. Tidsskr. **2:** 464.

Lyngbye, H. C. 1819, Tentamen Hydrophytologiæ Danicæ. Hafniæ.

Magdeburg, P. 1933, Organogene Kalkkonkretionen in Höhlen. Sitzungsber. d. naturf. Ges. Leipzig **59**.

Mainx, F. 1928, Einige neue Chlorophyceen. Arch. f. Protistenkunde **64:** 93.

— 1929, Untersuchungen über den Einfluss von Aussenfaktoren auf die phototaktische Stimmung. Arch. f. Protistenk. **68:** 105.

Massart, J. 1891, Recherches sur les organismes inférieurs III. La sensibilité à la gravitation. Bull. acad. roy. de Belgique 3. sér. **22:** 158.

Matruchot et Molliard 1900, Variations de structure d'une algue verte Stichococcus bacillaris Näg. sous l'influence du milieu. C. R. acad. sc. Paris **131**.

— 1902, Variations de structure etc. Rev. gén. de Bot. **14:** 193.

Meinhold, T. 1911, Beiträge zur Physiologie der Diatomeen. Beitr. z. Biol. d. Pfl. **10:** 353.

Mendrecka, S. 1913, Étude sur des algues saprophytes. Bull. soc. bot. Genève 2. sér. **5:** 150.

Meneghini, G. 1838, Cenni sulla organographia e fisiologia delle alghe. Padova.

Mez, C. 1905, Einige pflanzengeographische Folgerungen aus einer neuen Theorie über das Erfrieren Eis-beständiger Pflanzen. Engl. Jahrb. **34**, Beibl. 79: 40.

Miquel, P. 1892, De la culture artificielle des Diatomées. Le Diatomiste **1:** 73.

Molisch, H. 1926, Pflanzenbiologie in Japan. Jena.

Moore and Carter 1926, Further studies on the subterranean algal flora of the Miss. Bot. Garden. Ann. Miss. Bot. Gard. **13:** 101.

Moore and Karrer 1919, A subterranean algal Flora. Ann. Miss. Bot. Gard. **6:** 281.

Müller, O. 1899, Bacillariaceen aus den Natronthälern von El Kab. Hedwigia **38:** 274.

— O. Fr. 1767, Flora Fridrichsdalina. Argentorati.

Muenscher, W. C. 1923, Protein synthesis in Chlorella. Bot. Gaz. **75:** 249.

Nägeli, C. 1849, Gattungen einzelliger Algen. Zürich.

Nienburg, W. 1916, Die Perzeption des Lichtreizes bei den Oscillarien und ihre Reaktion auf Intensitätsschwankungen. Zeitschr. f. Bot. **8:** 161.

Oltmanns, F. 1917, Über Phototaxis. Zeitschr. f. Bot. **9:** 257.

Pascher, A., 1925, Heterokontæ etc. in Die Süsswasserflora Deutschlands etc. Heft 11.

Petersen, J. Boye 1915, Studier over danske aërofile Alger. Det kgl. danske Vid. Selsk. Skr. 7. Række, Math.-naturv. Afd. **12**, Nr. 7.

— 1923, The freshwater Cyanophyceæ of Iceland. The Bot. of Iceland **2**, Nr. 7.

Petersen, J. Boye, 1928 I, The aërial algæ of Iceland. The Bot. of Iceland **2:** No. 8.

— 1928 II, Algefloraen i nogle Jordprøver fra Island. Dansk Bot. Arkiv **5:** Nr. 9.

— 1932 I, The algal vegetation of Hammer Bakker. Bot. Tidsskr. **42:** 1.

— 1932 II, Einige neue Erdalgen. Arch. f. Protistenk. **76:** 395.

— 1932 III, Über das Wachstum von Erdalgen. Planta **17:** 15.

Petrová, J. 1931, Die vermeintliche Heterokonte "Botrydiopsis" — eine Chlorophycee. Beih. z. Bot. Centralbl. I, **48:** 221.

Pfeffer, W. 1884, Locomotorische Richtungsbewegungen durch chemische Reize. Untersuch. Bot. Inst. Tübingen **1:** 363.

— 1888, Über chemotaktische Bewegungen von Bacterien, Flagellaten und Volvocineen. Untersuch. Bot. Inst. Tübingen **2:** 582.

— 1904, Pflanzenphysiologie, 2. Aufl. Bd. II, Leipzig.

Pfitzer, E. 1882, Die Bacillariaceen in Schenk: Handbuch der Botanik **2.** Breslau.

Pieper, A. 1915, Die Phototaxis der Oscillarien. Diss. Berlin.

Piercy, A. 1917, The structure and mode of life of a form of Hormidium flaccidum A. Br. Ann. of Bot. **31:** 513.

Pringsheim, E. G. 1912 I, Die Reizbewegungen der Pflanzen. Berlin.

— 1912 II, Kulturversuche mit chlorophyllführenden Mikroorganismen I. Beitr. Biol. Pflanzen **11:** 305.

— 1913, Kulturversuche mit chlorophyllführenden Mikroorganismen II, III. Beitr. Biol. Pfl. **12.**

— 1914, Kulturversuche mit chlorophyllführenden Mikroorganismen IV. Beitr. Biol. Pfl. **13.**

Printz, H. 1914, Kristianiatraktens Protococcoideer. Vidensk. Selsk. Skr. I Math.-Naturv. Kl. 1913, No. 6.

— 1927, Chlorophyceae in Engler und Prantl: Die natürlichen Pflanzenfamilien II Aufl., Bd. 3. Leipzig.

Prowazek, S. 1900, Synedra hyalina, eine apochlorotische Bacillarie. Österr. bot. Zeitschr. **50:** 69.

Puymaly, A. de 1924, Recherches sur les algues vertes aériennes. Diss. Bordeaux.

Rabenhorst, L. 1864—68, Flora europæa algarum aquæ dulcis et submarinæ. I—III. Lipsiæ.

Radais 1900, Sur la culture pure d'une algue verte; formation de chlorophylle à l'obscurité. C. R. acad. sc. Paris **130:** 793.

Ramann, E. 1911, Bodenkunde. 3. Aufl. Berlin.

Richter, A., und Orlowa, K. 1928, Quantitative Feststellung der Algenvegetation in den Böden bei Saratow. Journ. f. Landw. Wissensch. Moskau **5:** 315 (not seen).

Richter, L. 1899, Zur Frage der Stickstoffernährung der Kulturpflanzen. Landw. Vers.-Stat. **51:** 221.

Richter, O. 1906, Zur Physiologie der Diatomeen. Sitzungsber. Wien. Akad. (I) **115:** 27.

— 1911, Die Ernährung der Algen. Monogr. u. Abh. zur Intern. Revue der ges. Hydrobiol. und Hydrogr. **2.**

Richter O. 1913, Die Reinkultur und die durch sie erzielten Fortschritte, vornehmlich auf botanischen Gebiete. Progressus rei botanicæ **4:** 301.

Robbins, W. W. 1912, in Colorado agr. Exp. Sta. Bull. 184 (not seen).

Rothamsted experimental Station 1925. Journ. of Bot. **63:** 366.

Russell, J. 1923, The Micro-Organisms of the soil. London.

Schloesing et Laurent 1891, Sur la fixation de l'azote libre par les plantes. C. R. acad. Paris **113:** 776.

— — 1892 I, Sur la fixation de l'azote libre par les plantes. C. R. acad. Paris **115:** 659.

— — 1892 II, Recherches sur la fixation de l'azote libre par les plantes. Ann. Inst. Pasteur **6:** 65.

Schmid, G. 1918, Zur Kenntnis der Oscillarienbewegung. Flora **111—112:** 327.

— 1921, Über Organisation und Schleimbildung bei Oscillatoria jenensis. Pringsh. Jahrb. f. wiss. Bot. **60:** 572.

— 1923, Das Reizverhalten künstlicher Teilstücke, die Kontraktilität und das osmotische Verhalten der Oscillatoria jenensis. Pringsh. Jahrb. f. wiss. Bot. **62:** 328.

Schmidle, W. 1901, Über drei Algengenera. Ber. d. d. bot. Ges. **19:** 10.

Schmidt, J. 1899, Danmarks blaagrønne Alger I, Hormogoneæ. Bot. Tidsskr. **22:** 283.

Schramm, J. R. 1914, A contribution to our knowledge of the relation of certain species of grass-green Algae to elementary nitrogen. Ann. Miss. bot. Gard. **1:** 157.

Schröder, G. 1886, Über die Austrocknungsfähigkeit der Pflanzen. Untersuch. Bot. Inst. Tübingen **2:** 1.

Schumacher, C. F. 1801—03, Enumeratio plantarum in partibus Sællandiæ septentrionalis et orientalis. Hafniæ, I, II.

Schwarz, F. 1884, Der Einfluss der Schwerkraft auf die Bewegungsrichtung von Chlamidomonas und Euglena. Ber. d. d. bot. Ges. **2:** 51.

Singh, H. D. 1933, A contribution to our knowledge of the algal flora of Lahore soils. Journ. of the Ind. bot. Soc. **12:** 102.

Smith, W. 1853—56. Synopsis of the british Diatomaceæ. London.

Stahl, E. 1880, Über den Einfluss von Richtung und Stärke der Beleuchtung auf einige Bewegungserscheinungen im Pflanzenreiche. Bot. Zeit. **38:** 297 etc.

— 1884, Zur Biologie der Myxomyceten. Bot. Zeit. **42:** 145.

Strasburger, E. 1878, Wirkung des Lichtes und der Wärme auf Schwärmsporen. Jena.

Strøm, K. M. 1928, Active reaction of the medium and the growth of Hormidium forms. Bull. Soc. bot. Genève **20:** 1.

Tischutkin, N. 1897, Über Agar-agarkulturen einiger Algen und Amöben. Centralbl. f. Bakt. II, **3:** 183.

Treboux, O. 1905, Organische Säuren als Kohlenstoffquelle bei Algen. Ber. d. d. bot. Ges. **23:** 432.

Treub, M. 1888, Notice sur la nouvelle Flore de Krakatau. Ann. du Jard. bot. de Buitenzorg. **7:** 211.

Van Heurck, H. 1899, Traité des Diatomées. Anvers.

— 1880—85, Synopsis des Diatomées de Belgique. Anvers.

Vaucher, J.-P. 1803, Histoire des Conferves etc. Genève.

Verplancke, G. 1932, L'examen microbiologique du sol. Mededeel. Bot. Inst. d. Rijks Univ. Gent, **1**.

Verworn, M. 1889, Psycho-physiologische Protisten-Studien. Jena.

Vischer, W. 1926, Études d'Algologie expérimentale. Bull. d. l. soc. bot. de Genève 2. sér. **18:** 184.

— 1933, Über einige kritische Gattungen und die Systematik der Chaetophorales. Beih. z. Bot. Centralbl. **51:** 1.

Waksman, S. A. 1931, Principles of soil Microbiology. 2. ed. London.

Walz, J. 1866, Beitrag zur Morphologie und Systematik der Gattung Vaucheria D. C. Pringsh. Jahrb. f. wiss. Bot. **5:** 127.

— 1868, Beitrag zur Kenntnis der Zoosporenbildung bei den Algen. Bot. Zeit. **26:** 497.

Wann, F. B. 1921, The fixation of free nitrogen by green plants. Am. Journ. Bot. **8:** 1.

Warming, E. 1904, Bidrag til Vadernes, Sandenes og Marskens Naturhistorie. Kgl. danske Vid. Selsk. Skr. 7 Række, Math.-naturv. Afd. II, 1.

— 1906, Dansk Plantevækst. 1. Strandvegetationen. København.

Warming und Graebner 1918, Lehrbuch der ökologischen Pflanzengeographie. 3. Ausg. Berlin.

West, W. and G. S. 1904—23. A Monograph of the British Desmidiaceæ I—V. London.

West, G. S. and Starkey, C. 1915, A contribution to the cytology and life history of Zygnema ericetorum (Kütz.) Hansg. etc. New Phytol. **14:** 194.

Wettstein, F. von 1921, Zur Bedeutung und Technik der Reinkultur für Systematik und Floristik der Algen. Österr. bot. Zeitschr. **70:** 23.

Wilfarth und Wimmer 1907, Über den Einfluss der Mineraldüngung auf die Stickstoffbindung durch niedere Organismen im Boden. Landw. Vers. Stat. **67:** 28.

Wille, N. 1911, Nachträge zum I Teil, 2 Abt. in Die natürlichen Pflanzenfamilien von Engler und Prantl.

— 1925, Vorarbeiten zu einer Monographie der Chroococcaceen, herausgegeben von K. Münster Strøm. Nyt Magaz. f. Naturv. **62:** 169.

Winogradsky, S. 1925 I, Sur une méthode pour apprécier le pouvoir fixateur de l'azote dans les terres. C. R. Acad. sc. Paris **180:** 711.

— 1925 II, Études sur la microbiologie du sol. Ann. Inst. Pasteur **39:** 299.

Wislouch, S. M. 1910, Über das Ausfrieren (Kältetod) der Alge Stichococcus bacillaris Näg. unter verschiedenen Lebensbedingungen. Bull. Jard. imp. bot. St. Pétersbourg **10:** 166.

Zumstein, H. 1900, Zur Morphologie und Physiologie der Euglena gracilis Klebs. Pringsh. Jahrb. f. wiss. Bot. **34:** 149.

Østrup, E. 1901, Freshwater Diatoms from the Færoës. Bot. of the Færoës I.

— 1910, Danske Diatomeer, København.